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Agrovoc descriptors: steinernema feltiae; heterorhabditis; thripidae; thysanoptera; efficiency; mortality; thrips (genus); larvae; environmental factors

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First results concerning the efficacy of entomopathogenic nematodes against *Hercinothrips femoralis* (Reuter)

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ABSTRACT

The efficacy of the entomopathogenic nematodes *Steinernema feltiae* and *Heterorhabditis bacteriophora* against larvae and adults of banded greenhouse thrips, *Hercinothrips femoralis*, was studied under laboratory conditions. The activity of the biological agents under study was determined at three different temperatures (15, 20, and 25°C), with concentration of 200 infective juveniles per individual (larva or adult), a day:night ratio of 4:20 and relative humidity of 95 %. The experiment was conducted in plastic rearing vessels on French bean leaves with a slightly modified version of a method used for studying the bionomics of thrips. The mortality rate of the thrips was determined four days after the application of the nematode suspension. Temperature had significant influence on adult pest mortality, but no significant effects were found with nematode species. Neither temperature nor nematode species had significant effect on larval mortality, which ranged from 23 % (*S. feltiae* at 25°C) to approximately 50 % (*H. bacteriophora* at 15 and 25°C). Mortality of adults was significantly influenced only by temperature, with the nematodes being most efficient at 25°C (approximately 30 % mortality by *H. bacteriophora*). The results of our research showed that foliar application of entomopathogenic nematodes might be a relatively efficient way for controlling *H. femoralis*, but the optimization of environmental factors would likely improve their efficacy further.

Key words: Thysanoptera, Terebrantia, Thripidae, Panchaetothripinae, *Steinernema feltiae*, *Heterorhabditis bacteriophora*, efficacy

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IZVLEČEK

PRVI REZULTATI UČINKOVITOSTI ENTOMOPATOGENIH OGORČIC ZA ZATIRANJE
RESARJA *Hercinothrips femoralis* (Reuter)

V laboratorijskih razmerah smo preučevali učinkovitost entomopatogenih ogorčic *Steinernema feltiae* in *Heterorhabditis bacteriophora* za zatiranje ličink in odraslih osebkov resarja *Hercinothrips femoralis*. Delovanje ogorčic na žuželko smo ugotavljali pri treh različnih temperaturah (15, 20 in 25°C), koncentraciji 200 infektivnih ličink/osebke, razmerjem med svetlobo in temo 4:20 in 95% relativni zračni vlagi. Poskus smo izvajali v plastičnih gojitvenih posodicah na fižolovih listih, z rahlo modificirano metodo, ki je bila primarno razvita za raziskave bionomije resarjev. Štiri dni po aplikaciji suspenzije ogorčic smo ugotavljali smrtnost izpostavljenih resarjev. Med vrstama ogorčic nismo ugotovili significantnih razlik v delovanju na škodljivca. Vrsta ogorčic in temperatura nista imeli significantnega vpliva na smrtnost ličink, ki je znašala od 23% (*S. feltiae* pri 25°C) do približno 50% (*H. bacteriophora* pri 15 in 25°C). Smrtnost odraslih osebkov je bila significantno determinirana le s temperaturo, pri čemer so bile ogorčice najbolj učinkovite pri 25°C (približno 30% smrtnost pri *H. bacteriophora*). Rezultati naše raziskave kažejo, da je lahko foliarna aplikacija preučevanih biotičnih agensov relativno učinkovit način zatiranja resarja *H. femoralis*, z optimiziranjem okoljskih dejavnikov pa je mogoče njihovo učinkovitost še izboljšati.

Ključne besede: Thysanoptera, Terebrantia, Thripidae, Panchaetothripinae, *Steinernema feltiae*, *Heterorhabditis bacteriophora*, učinkovitost

1 INTRODUCTION

Banded greenhouse thrips, *Hercinothrips femoralis* (Reuter) (Thysanoptera: Thripidae), belong to a group of some ten Thysanoptera species which have been proven to be noxious to both cultivated and indigenous plants. Thrips are found across Africa, Central and North America and in Europe (Palmer *et al.*, 1989; Simon, 1993). On the Old Continent and in other areas with moderate climate, these pests are particularly noted in greenhouses (Lacasa in Martinez, 1988; Tusnadi in Nemstothy, 1992; Vierbergen, 1995; Reiderne *et al.*, 1997; Pintureau *et al.*, 1999; Nedstam, 2001; Trdan, 2002).

Although *H. femoralis* is known under the common name of sugar beet thrips, it is found almost everywhere where bananas are grown (Lewis, 1997) and also causes economically important damage to a variety of ornamental plants. In fact, it can feed on more than 50 host plants including representatives of the genera *Amaryllis*, *Aralia*, *Begonia*, *Chrysanthemum*, *Croton*, *Dieffenbachia*, *Dracaena*, *Ficus*, *Gardenia*, *Hydrangea*, *Philodendron*, *Schefflera*, *Schlumbergera* and others (Beshear, 1979; Oetting and Beshear, 1980; Nedstam, 2001). The sucking of the larvae and adults results in the appearance of silver spots and the aesthetic appearances of the attacked plants are further diminished due to the presence of black excrement.

Rare literature sources which deal with the chemical control of *H. femoralis* (Scarpelli and Bosio, 1999) show that these thrips are not tolerant to insecticides. Thus it is possible to use insecticides to reduce the number of the pest quickly and efficiently before they can spread to a greater extent. Recently, however, more and more importance has been assigned to finding environmentally acceptable ways of controlling plant pests (including thrips) – and a major line of research is the use of their natural enemies. Among these natural enemies are the entomopathogenic

nematodes (EPNs); soil organisms which are mutually associated with bacteria of the family Enterobacteriaceae. The nematodes enter the prey through their body openings. After infection, the EPNs' symbiotic bacteria are released into the insect hemocoel, causing septicemia and death of the insect (Kaya and Gaugler, 1993).

Most previous research employing EPNs has been directed against soil pests (Kaya et al., 2006). Yet increasing research utilizes their application more broadly and in some cases, EPNs have already been used practically. One of these productive lines of research is the foliar application of EPNs with which a faster activity of nematodes can be attained. Amongst the Thysanoptera species, most previous research has been focussed on western flower thrips (*Frankliniella occidentalis* [Pergande]) (Arthurs and Heinz, 2006; Shapiro-Ilan *et al.*, 2006), which is one of the four most harmful thrips species in the world (Kirk and Terry, 2003).

The aim of our research was to determine the efficacy of EPNs *Steinernema feltiae* and *Heterorhabditis bacteriophora* against the larvae and adults of *H. femoralis*. Differences in efficacy were expected between the temperatures and nematode species. Additionally, we also expected differences in susceptibility of thrips according to their developmental stages.

2 MATERIAL AND METHODS

2.1 Entomopathogenic nematodes and banded greenhouse thrips

A laboratory investigation was carried out in the Entomological Laboratory of the Chair of Entomology and Phytopathology (University of Ljubljana, Biotechnical Faculty, Department of Agronomy) in Ljubljana, Slovenia. Commercial biopreparations of *Steinernema feltiae* (Filipjev) (Rhabditida: Steinernematidae) and *Heterorhabditis bacteriophora* Poinar (Rhabditida: Heterorhabditidae) from Koppert B. V. (Berkel en Rodenrijs, The Netherlands) were used in the work. Both biopreparations, which were sent by air-mail, were used within 2 months of their receipt.

Hercinothrips femoralis was reared in the insectarium at room temperature and without artificial light. They were reared on young plants of *Mirabilis jalapa* and on the leaves of French bean (*Phaseolus vulgaris* L.). The suitability of the latter plant for rearing this thrips species has already been demonstrated (Takrony, 1973), but the rearing of the thrips on both hosts was relatively simple compared to the older rearing method of this insect (Laughlin, 1971). On both hosts, thrips had more numerous progeny than on a previous tested composition of *Chlorophytum comosum* and the orchids *Oncidium* Gower Ramsey and *Epidendrum* 'Ballerina yellow'. Adults and larvae of the pest were used for the laboratory research.

2.2 Laboratory bioassay

Suspensions of EPNs were prepared in glass jars. The efficacy of agents was tested using a concentration of 200 infective juveniles (IJs) per individual (larva or adult) or 1000 IJs in 1 ml of water. Transparent plastic vessels (10 x 10 x 3.5 cm) were used for the rearing of thrips. This rearing method has been used before to study the bionomics of thrips (Trdan, 2000). In contrast to the original method, no pollen was added to the bean leaves in the present study. Five thrips adults (or larvae) were transferred onto each leaf using a fine-tip brush. Following this, 1 ml of nematode suspension was added to each leaf, whereas in the control condition 1 ml of water without nematodes was added to the leaves. Suspensions were added using a pipette, with the tip being changed after every treatment. The plastic vessels were then closed.

The plastic vessels were put in a rearing chamber (RK-900 CH type from Kambič Laboratory equipment, Semič, Slovenia) with working capacity of 0,868 m³ (width x height x depth = 1000 x 1400 x 620 mm), and with every treatment in 5 replications. Efficacy was tested at three different temperatures (15, 20, and 25°C), but with constant light:dark ratio of 4:20 and relative humidity of 95%. The number of dead individuals was determined 4 days after treatment.

2.3 Statistical analysis

A multifactor analysis of variance (ANOVA) was conducted to determine the differences in mortality rates (%) between the larvae and adults of banded greenhouse thrips, reared in three different treatments (two species of EPNs and untreated control) at three different temperatures. Before the analysis, each variable was tested for homogeneity of treatment variances. The mortality rate data were corrected for control mortality according to Abbott's formula (Abbott, 1925) and the data were normalized by an arcsine square-root transformation before the analysis. Duncan's multiple range test ($P \leq 0.05$) was used to separate mean differences among the parameters in all the treatments. All statistical analyses were performed with Statgraphics Plus for Windows 4.0 (Statistical Graphics Corp., Manugistics, Inc., Maryland, USA) and figures were created with MS Office Excel 2003 (MS Corporation). The data are presented as untransformed means \pm SE.

3 RESULTS

With general analysis of variance it was determined that temperature ($P < 0.0498$) and developmental stage of the pest ($P < 0.0002$) had significant effects on mortality rate of *H. femoralis*, while no significant effects were seen with nematode species ($P < 0.1337$), interaction between EPN species and temperature ($P < 0.3412$), interaction between EPN species and developmental stage ($P < 0.2202$), interaction between temperature and developmental stage of the pest ($P < 0.3821$) and interaction between all three factors ($P < 0.8940$). With average mortality of $37.68 \pm 4.44\%$, larvae were more susceptible to nematode attack than were adults, which showed a mortality rate of $15.40 \pm 3.31\%$. Significantly the lowest mortality of thrips was seen at 20°C (16.88 ± 4.65), and between 15 ($30.89 \pm 6.12\%$) and 25°C ($31.85 \pm 4.86\%$) no respective significant differences were ascertained. *H. bacteriophora* showed the highest efficacy rates against thrips ($30.71 \pm 4.75\%$), but this was not significantly higher than the mortality rate caused by *S. feltiae* (22.37 ± 3.94).

Individual analysis of variance did not confirm any influence of EPN species ($P < 0.1151$), temperature ($P < 0.5276$), or their interaction ($P < 0.5220$) on the mortality of thrips larvae. Larval mortality ranged from just over 23 % (*S. feltiae* at 25°C) to approximately 50 % (*H. bacteriophora* at 15 and 25°C) (Figure 1).

Temperature had significant influence ($P < 0.0049$) on adult pest mortality, but no significant effects were found with EPN species ($P < 0.7693$) and its interaction with temperature ($P < 0.5696$). The lowest efficacy of both biological agents was found to occur at 15°C (about 17 % mortality of adults) and at 20°C (about 5 % mortality of adults), beside that between 15°C (about 15 % mortality of adults at both nematode species) and 25°C (about 30 % mortality of adults treated with *H. bacteriophora*) no significant differences were determined (Figure 2).

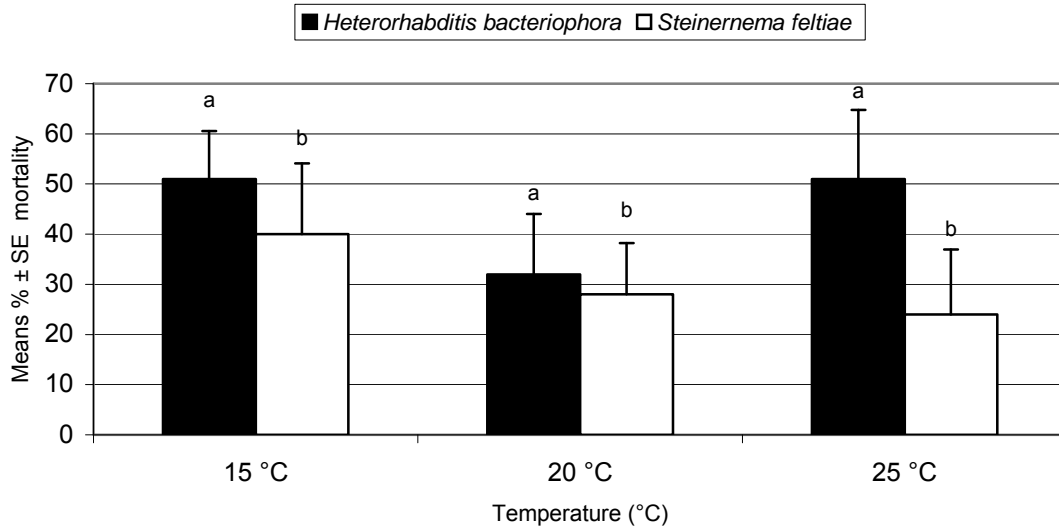


Figure 1: Mean larval mortality (\pm SE) of *Hercinothrips femoralis* treated with two different species of entomopathogenic nematodes (200 IJs/individual) depending on rearing temperature. Data shown are corrected for control mortality and analyzed by multifactor ANOVA. Mean values followed by the same letter do not differ significantly ($P \leq 0.05$) according to Duncan's multiple range test. The letters correspond to the grouping of means for temperature, while for EPN species no significant differences were established.

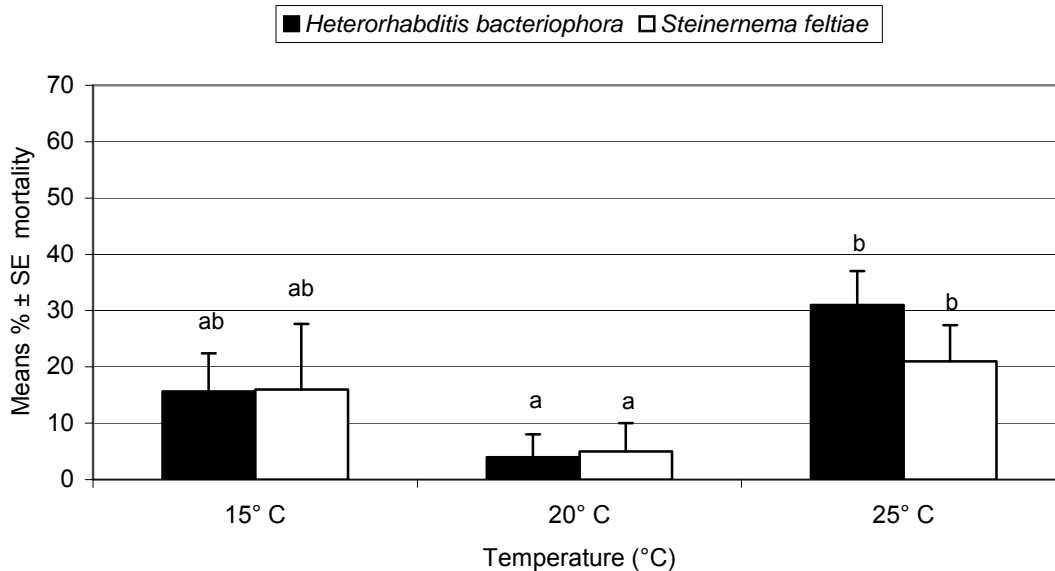


Figure 2: Mean adult mortality (\pm SE) of *Hercinothrips femoralis* treated with two different species of entomopathogenic nematodes (200 IJs/individual) depending on rearing temperature. Data shown are corrected for control mortality and analyzed by multifactor ANOVA. Mean values followed by the same letter do not differ significantly ($P \leq 0.05$) according to Duncan's multiple range test. The letters correspond to the grouping of means for temperature, while for EPN species no significant differences were established.

4 DISCUSSION

Though the first record of *H. femoralis* in Slovenia on some of the ornamental plants was expected (Trdan and Vierbergen, 2001), it was somewhat surprising when it was found on young maize plants grown in the laboratory (Trdan, 2002). Some sources (*Hercinothrips...*, 2002) quoted maize as one of the potential hosts of banded greenhouse thrips. The thrips most likely entered into the laboratory with some ornamental plants, and remained in the same laboratory since 2002 or even earlier.

The fact that indoor work and living places are important habitats of banded greenhouse thrips has implications for the control methods that can be used. Special attention should be paid to guard against secondary damage to human comfort or health. With the choice of some of the environmentally acceptable control methods, we may be able to circumvent some of the problems associated with chemical pesticides. Among these methods we include EPNs, whose efficacy against Thysanoptera species has been particularly studied with western flowers thrips in the greenhouses. Higher efficacy of EPNs against this pest has been shown with a soil application of the suspension (Buitenhuis and Shipp, 2005), with earlier and multiple applications at lower concentration (Belay *et al.*, 2005), at higher soil moisture (Ebssa *et al.*, 2004a) and temperatures of 25°C (Ebssa *et al.*, 2004b).

Foliar applications of EPNs on plants attacked by thrips represents one of the more recent potential methods of their use. Although previous results of such research do not lend strong support to foliar application of EPNs (compared to soil application), particularly due to their sensitivity to desiccation and UV radiation (Shapiro-Ilan *et al.*, 2006), this method could nevertheless be suitable for the control of thrips on (tropical) plants, which often require water applications to leaves and which preferentially grow in places not exposed to direct sunlight. In fact, many hosts of *H. femoralis* like such habitats.

In our research, the larvae of banded greenhouse thrips were more sensitive to nematode infection than were adults. This confirms the known fact that EPNs are most efficient against the larvae and other preimaginal stages of insects, as they can enter their bodies more easily (LeBeck *et al.* 1993). For this reason, a weaker activity associated with foliar applications *S. feltiae* and *H. bacteriophora* on adults of *H. femoralis* is no surprise. Furthermore, in recent related research on adults of western flower thrips, similar conclusions were reached (Buitenhuis and Shipp, 2005). A poorer efficacy of the nematodes in our research might also be attributed to lower concentration of suspension (200 infective juveniles/individual), which was applied to bean leaves only once. It is often necessary to apply such a suspension repeatedly in order to achieve satisfactory efficacy of these biological agents (Belay *et al.*, 2005), and in this particular detail we see a chance to notably improve the efficacy against *H. femoralis* on ornamental plants via repeated EPN foliar applications.

Temperature had no influence on nematode efficacy against larvae, but this was not the pattern for adults where the mortality was significantly higher at 25°C. This is in accordance with nematode efficacy in controlling western flower thrips (Ebssa *et al.*, 2004b). We attribute the weak activity of the nematodes against the banded

greenhouse adults at 20°C to the highest vitality of thrips at this temperature, most likely promoting its natural resistance to infection with EPNs.

H. femoralis also survives on above-ground parts of host plants during pre-pupal and pupal stages, especially on older leaves (Oetting *et al.*, 1993). Foliar application of the nematodes is therefore a legitimate method of pest control. We note that western flower thrips and many other damaging Thysanoptera species (Tommasini and Maini, 1995) pupate in the soil. Furthermore, thrips in pre-pupal and pupal stages are especially susceptible to EPN infection (Ebssa *et al.*, 2001) and are almost immobile.

Thus these first results concerning the control *H. femoralis* with foliar applications of EPNs showed that these biological agents could be, along with optimal abiotic and biotic factors, an effective enough alternative to the current prevailing (conventional) methods of pest control. Therefore, further research from our group will be oriented to the optimization of foliar application methods of EPNs to control all above-ground developmental stages of the pest.

5 ACKNOWLEDGEMENT

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Agrovoc descriptors: brassica oleracea; spacing; thrips (genus); thrips tabaci; crop yield; damage; quality; dimensions; crop losses

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Yield and quality of early cabbage (*Brassica oleracea* L. var. *capitata*) in response to within-row plant spacing

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ABSTRACT

The plant spacing of early cabbage (*Brassica oleracea* L. var. *capitata*) was studied at the Experimental Field of the Biotechnical Faculty in Ljubljana, during the 2001 and 2002 growing season. The cultivars, which included 'Vestri', 'Parel', 'Delphi', 'Destiny' and 'Hermes', were each spaced at 20, 30 and 40 cm within rows and 30 cm between rows or populations equivalent to 166,000, 108,000 and 82,000 plants ha⁻¹. The experimental variables measured were marketable yield (per head and per hectare), yield characteristics (head diameter and head volume), quality characteristics (core volume, head density, soluble solids and dry matter) and onion thrips damage ratings. There was no interaction effect of cultivar and planting spacing on the measured variables. Marketable yield per head, head diameter and volume, core volume and head density generally increased as the within-row plant spacing increased, whereas, dry matter was significantly decreased at lower plant spacing. Soluble solids were not affected by plant spacing. The yield potential of cabbage was higher at cv. 'Vestri' than at the other cultivars. The onion thrips damage rating was severe at the lowest plant spacing and contributed to the reduced yield. A higher damage rating was established in the cv. 'Parel' but this didn't have a statistically significant influence on the weight loss of yield.

Key words: *Brassica oleracea*, cabbage, yield, head quality, onion thrips, damage

IZVLEČEK

PRIDELEK IN KAKOVOST ZGODNJEGA ZELJA (*Brassica oleracea* L. var. *capitata*) V ODVISNOSTI OD RAZDALJE V VRSTI

Poskus s sadilnimi razdaljami zgodnjega zelja (*Brassica oleracea* L. var. *capitata*) je bil opravljen na Laboratorijskem polju Biotehniške fakultete v letih 2001 in 2002. Medvrstna razdalja za pet kultivarjev zelja ('Vestri', 'Parel', 'Delphi', 'Destiny' in 'Hermes') je znašala 30

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cm, medtem ko so bile razdalje v vrsti 20, 30 in 40 cm, kar, preračunano na površino enega ha, pomeni 166.000, 108.000 oz. 82.000 rastlin. Ugotavljali smo tržni pridelek (na glavo in na ha), lastnosti pridelka (premer glave in prostornino glave), kvalitativne lastnosti (prostornino vretena, gostoto glave, suho snov in sušino) in ocenili poškodbe, ki jih je povzročil tobakov resar. Med dejavnikoma kultivar in gostota sajenja ni prišlo do interakcije, ki bi vplivala na opazovane lastnosti. Tržni pridelek glav, premer in prostornina glav, prostornina vreten in gostota glav so na splošno naraščali z večanjem razdalje v vrsti, medtem ko se je pri najmanjši gostoti sušina značilno zmanjšala. Sadilna razdalja ni vplivala na vsebnost suhe snovi. Cv. 'Vestri' se je v primerjavi z preostalimi kultivarji izkazal kot kultivar z možnostjo doseganja najvišjih pridelkov. Poškodbe, ki jih je povzročil tobakov resar, so bile največje pri najmanjši sadilni razdalji in so bile razlog za zmanjšanje pridelka. Največ poškodb je pretrpel cv. 'Parel', a to ni statistično značilno vplivalo na izgubo pridelka.

Ključne besede: *Brassica oleracea*, zelje, pridelek, kakovost glav, tobakov resar, poškodbe

INTRODUCTION

Cabbage (*Brassica oleracea* L. var. *capitata*) is a typical vegetable crop of Slovenia with a cultivated area of around 450 ha, which represents 20.1% of the field in production in this country (Statistical Office RS, 2005). Slovenia has the favourable agroclimatic conditions for the production of cabbage varieties for fresh market and for processing, namely.

Plant population studies are common features of many horticultural crops, including cabbage (Stoffela and Bryan, 1988). There are several citations in the literature that provide information relative to optimum plant population evaluations for cabbage that been conducted over the past 30 years (Day, 1986; Stoffela and Fleming, 1990; Lal, 1996; Stepanović et al., 2000). A very common range in optimum plant population recommendations for cabbage is for stand densities of 20,000 to 70,000 plants per ha (Ghanti et al., 1982; Tendaj and Kuzyk, 2001; Kumar and Rawat, 2002). But results of these reports have been inconclusive. Cabbage yield and quality response to plant density has been shown to be influenced by several factors such as the plant genotype, climate condition, soil, water regime, nutrient status, market requirements and many others (Knavel and Herron, 1981; Parmar et al., 1999; Tiwari et al., 2003).

Increasing plant population with cabbage has the potential for increasing yield and profit. Although in cabbage, high plant density reduced head size and head weight (Csizinsky and Schuster, 1985) a greater number of heads per unit area increased total yield (Stepanović et al., 2000).

Though many authors have reported some information on the plant density of different varieties of cabbage, studies under Slovenia agro climatic conditions are limited. There were two major objectives to this experiment. First we estimated which plant spacing and which variety are optimal for cabbage production. Second, we conducted to determine whether varieties differ with respect to their susceptibility of resistance to onion thrips (*Thrips tabaci* Lindeman) which is prevalent in Slovenian cabbage fields.

MATERIAL AND METHODS

Location and climate conditions

The observation were made over two years at the Experimental Field (46° 04' N, 14° 31' W, 300 m above sea level) of the Biotechnical Faculty in Ljubljana, Slovenia. The climate at the site is characterized by typical temperate continental climate. The average growing period rainfall is 527.9 mm and cumulated temperatures were 341.2 °C.

Primary weather data for the experimental period were obtained from the meteorological station located in the experimental field, which is situated at distance of 500 m from the experimental plot. Each of the experimental years was warmer than the long term average, although in 2001, the temperature in April was lower. The 2001 growing period was relatively hotter than the 2002 period. Between 10 July and 20 July, the maximum temperature was 24.6 °C in 2001 as compared to 20.7 °C in 2002. The total precipitation received in 2001 was relatively lower than in the long-term average, but there were some heavy rains in May. However, crop growth was not affected by drought because to supplement low precipitation, irrigation was applied several times during the growing period.

Table 1: Climatologically data for Ljubljana (Slovenia) during the experimental period

Month	Mean temperature (°C)			Sums of precipitation (mm)		
	2001	2002	Long-term ¹	2001	2002	Long-term ¹
April	17.1	14.7	17.2	71.7	90.7	117.5
May	18.3	21.1	17.8	147.3	175.6	148.4
June	21.9	21.3	19.9	32.6	127.0	121.8
July	22.9	20.1	19.1	89.6	186.6	140.2

¹ 30 years mean in Ljubljana, Slovenia

Experimental design

The experimental design was a split-plot within a randomized complete block design with three replications. The main plot treatments consisted of five early cabbage varieties, namely 'Vestri', 'Parel', 'Delphi', 'Destiny' and 'Hermes' and each subunit consisted of three spacing densities. Varieties which are widely grown by local growers were included in the experiment for comparison purposes. The above spacing resulted in plant population of 166,000 108,000 and 82,000 plants ha⁻², respectively. The constant distance between rows was 30 cm. Within-row spacing used were 20 cm, 30 cm and 40 cm. The experimental unit consisted of three rows within sixteen plants in each row.

Seeding was done in 72 cell trays in the greenhouse in mid-March. Plants were hardened-off prior to transplanting in the field. Transplanting about 30 days old seedlings to raised beds was done on April 17, 2001, and April 21, 2002. Black polyethylene mulch and drip tape (T-tape TSX 500 Model, T-systems International; 0.25-m emitter spacing) were used as typically done in commercial field in Slovenia. Fertilizer was applied preplant incorporated and during the season through drip emitter using water soluble 21-21-21 NPK. No insecticide was applied in order to see the effect of onion thrips resistance on yield and quality.

The heads were harvested from each treatment beginning when the variety appeared to be approaching horticultural maturity by the usual visual criteria. A sample of eight heads per treatment was cut and split longitudinally to measured the following parameters: marketable head weight, head polar and equatorial diameters and core length and base width. The head density was calculated as previously reported (Pearson, 1983), using the marketable weight of individual head and average of the equatorial and polar head diameters. The volumes of heads and cores were estimated using the sphere and cone formula, respectively (Kleiheinz and Wszelaki, 2003). The marketable head weight data was converted into values marketable

yield per hectare. Soluble solids concentration of the expressed juice was determined using a hand-held Atago PR1 refractometer. Head dry weight was determined by drying the leaves in oven at approximately 60 °C until a constant weight was reached.

After frame leaves were removed we also visual inspected the presence of onion thrips damage. The four heads per treatment was peeled to remove leaves damaged by thrips feeding. The amount of peeled leaves was weighed to indicate a measure of thrips tolerance. The leaves damaged was rated on severity scale of 1 (no damage) up to 6 (severe damage) (Stoner and Shelton, 1988). According Trdan et al. (2005) the scale was slightly modified, because additionally the interval between 26 and 50 % of damaged leaf surface was taken into consideration.

RESULTS AND DISCUSSION

Because there was no significant difference in interaction between variety and planting density for all measured variables, analyses of the results is focused on the differences within the main effects variables, which included variety and planting density. Significant differences are marked with different letters.

Influence of plant spacing on yield and yield characteristics

By mean values reported in Table 2, it emerges that head production attained highest yields in 2002. These conclusions can statistically confirm for marketable yield per head as well as per hectare. It is difficult to determine, however, the precise influence of environmental factors in this study on head formation. The temperature is primary variable for plant growth and development (Masaya et al., 2004). The results suggest the deleterious effect of hot weather conditions on cabbage head formation. Between 10 July and 20 July, 2001 the plants exposed to higher temperature for 3.9 °C in comparison the same period in 2002. At this stage of development (expansion of internal head leaves), the heads had produced more than 20% of their biomass. Generally, the temperatures above 24 °C are not suited to cabbage production (Kahn et al., 2007). These results are in agreement with those obtained by Radovich et al. (2004). These researches pointed out that temperature appears to be the factor contributing most to the variation in growth observed between years. It is not known however, what the critical duration of high temperature of exposure is required to cause limiting growth in cabbage.

As within-row spacing decreased yield per head increased. The highest marketable head weight obtained was 891.5 g from the lowest plant spacing (8.2 plant m⁻²). On the other hand no significant differences in marketable yield per hectare were found among highest and lowest plant spacing. Our results indicate that plants at the lower rate were able to compensate for the lower plant numbers by increasing head volume. For all plant spacing, there was a positive linear relationship between head weight and head volume (data not shown). The 'Vestri' produced the highest marketable yield per head and per hectare. A comparison of the head diameter of the seasons revealed quite different results. The trend was similar in head weight. A reduction in head weight was the cause for the difference in the head diameter. Head diameter generally increased with decreased plant spacing. Semuli (2005) mentioned that it is possible that as plant spacing was reduced, competition for nutrients, light, air and moisture increased which would have resulted in decreased diameter and weight of heads.

Stofella and Fleming (1990) reported quadratic increases in the cabbage head height and head width as intra-row spacing was increased from 8 to 38 cm. The lowest plant spacing resulted in significantly wider equatorial diameter than the polar one.

Table 2: Influence of plant spacing on marketable yield, head diameter and head volume

Treatment	Marketable yield		Head diameter (cm)		Head volume (cm ⁻³)
	head ⁻¹ (g)	ha ⁻¹ (t)	equatorial	polar	
<i>Year</i>					
2001	418.5 a	49.4 a	10.1 a	11.4 a	562.4 a
2002	862.6 b	101.7 b	11.2 b	13.0 b	783.7 b
<i>Cultivar</i>					
Vestri	810.8 a	95.6 a	11.0 b	12.0 a	750.0 b
Hermes	623.8 b	73.6 b	10.8 b	11.7 a	708.6 b
Delphi	629.3 b	74.3 b	10.2 a	12.0 a	582.4 a
Destiny	562.9 c	66.4 c	11.1 b	12.3 a	769.5 b
Parel	604.5 b	71.3 b	10.0 a	13.0 b	556.2 a
<i>Spacing (m⁻²)</i>					
16.6	428.0 a	71.0 a	9.6 a	11.2 a	480.5 a
10.8	602.5 b	65.0 b	12.7 b	12.0 b	678.4 b
8.2	891.5 c	73.1 a	16.6 c	13.1 c	860.5 c

Influence of plant spacing on quality characteristics

Plant spacing showed significant effects on quality characteristics (Table 3). Core volume was significantly increased by decreasing plant spacing. The highest core volume (43.7 cm³) was recorded in heads from the lowest spacing. It was found that the mean percent of core volume which occupied the head didn't varying among various levels of plant spacing. A comparison of percent of core volume per head among cultivars revealed a significant difference. Highest measurements for this parameter were obtained at 'Vestri' (7.6%) and lowest at 'Destiny' (3.2%).

The head density, a primary indicator of horticultural maturity (Radovich et al., 2004), appeared to be significantly influenced by plant spacing. Although Kleinheinz and Wszelaki (2003) reported that the head density generally exceed 0.70 g cm⁻³, in our experiment these values in some cases were lower. For example, the maximum plant spacing of 16.6 plants m⁻² was achieved minimum head density 0.62 g cm⁻³. Across the cabbage cultivars evaluated, 'Destiny' was achieved only 0.60 g cm⁻³.

The percentage of soluble solids was higher in the low plant spacing, followed by the medium spacing and the high plant spacing. But these values were not significantly affected.

The percent of dry matter, which consist both of soluble and insoluble carbohydrates, was significantly influenced by plant spacing. According Raupp (2000) percentage of dry matter is an important reference parameter, and is somewhat significant as well to a consumer who does not want to buy watery products. Heads grown at low spacing produced less dry matter than at high spacing. These results are in agreement with those obtained by Agele et al. (1999) on tomato and Siomos (1999) on pak choi. On the other hand, our results are not conformity with those of Koutsos and Koutsika-Sotiriou (2001) who mentioned that increasing the volume of heads decreased the dry matter percent. In 2002, heads produced cca 20% more dry matter than in 2001, probably due to the lower temperature in June.

Table 3: Influence of plant spacing on quality of cabbage

Treatment	Core volume		Head density (g cm ⁻³)	Soluble solids (%)	Dry matter (%)
	(cm ⁻³)	(% head ⁻¹)			
<i>Year</i>					
2001	17.1 a	3.4 a	0.70 a	5.8 a	7.8 a
2002	55.6 b	7.7 b	0.84 b	5.6 a	9.6 b
<i>Cultivar</i>					
Vestri	54.4 a	7.6 a	0.84 a	5.5 a	10.5 a
Hermes	35.1 b	4.9 c	0.73 b	5.0 a	9.0 b
Delphi	30.6 c	5.2 c	0.78 b	5.9 a	8.9 b
Destiny	54.4 a	3.2 d	0.60 c	5.4 a	10.4 a
Parel	36.8 b	6.8 b	0.78 b	5.2 a	7.2 c
<i>Spacing (m⁻²)</i>					
16.6	28.7 a	6.2 a	0.62 a	5.3 a	10.6 a
10.8	36.8 b	5.5 a	0.76 b	5.6 a	9.6 b
8.2	43.7 c	6.0 a	0.89 c	5.8 a	8.8 c

Influence of plant spacing on thrips damage

The thrips damage ratings of the head differed greatly (Table 4). The thrips pressure on cabbage was much larger in 2001 than in 2002. High temperatures during first year should be resulting in a greater extent of damage on the cabbage heads. Temperatures as high as 32 °C were recorded on several occasions during head formation in June and July. Thrips *tabaci*, which are polyphagous insects, much rather remain inside of cabbage heads where they are protected against sunburn. Because thrips remain inside of cabbage heads for longer in this type of weather than in the “mean year” (Tamo et al., 1993), the extent of their damage is greater. The other reason may have caused by the heavy rainy summer in 2002. The extreme population drop in early June (data not shown) corresponded to a heavy rainfall event. However, the trend of susceptibility of each variety's was similar in both years. In 2001 and 2002 we concluded the lowest damage in the ‘Vestri’, the highest in the ‘Parel’ and smaller statistical differences the

‘Hermes’, ‘Delphi’ and ‘Destiny’. These differences were more apparent in 2001 than in 2002. The lowest damage ratings was found in the most densely plant spacing (16.6 plants m⁻²) and the highest in the least densely planted category (8.2 plants m⁻²).

Table 4: Influence of thrips damage on heads characteristics

Treatment	Damage ratings	Peeled leaves	
		weight (g)	(% head ⁻¹)
<i>Year</i>			
2001	1.76 a	162.5 a	28.1 a
2002	1.33 b	235.7 b	20.9 b
<i>Cultivar</i>			
Vestri	1.33 a	168.8 a	19.3 a
Hermes	1.55 b	178.1 b	23.0 b
Delphi	1.65 b	217.3 c	27.6 c
Destiny	1.72 b	214.0 c	28.2 c
Parel	1.96 c	202.8 c	26.5 c
<i>Spacing (m⁻²)</i>			
16.6	1.42 a	111.6 a	22.8 a
10.8	1.65 b	185.1 b	25.0 b
8.2	1.85 c	300.7 c	28.7 c

The weight loss of yield (peeled leaves) due to onion thrips damage on the cabbage heads varied between the two years and was larger in 2001 when the cabbage was exposed higher attack of thrips tabaci. Similar results were obtained in terms of percent loss of yield. ‘Vestri’ clearly showed a tendency to have the least % loss of yield. Statistically significant differences were found also in the various plant spacing. In the highest density, we found the lowest % yield loss. On the contrary in the lowest density, this parameter was the highest.

CONCLUSIONS

Five white cabbage cultivars were evaluated in 2001 and 2002 for the best combination of planting spacing for advancing growth and for obtaining highest marketable yield and head quality. It should be noted that the experiment was stressed by unfavourable growing conditions in the 2001. Very high temperatures in first year led to some reduction in yield potential and enlarge ratings of damage of thrips tabaci.

At the widest spacing treatment the marketable yield per head is highest. Therefore, we can conclude that plant at more space growth can use efficiently water, nutrients and solar radiation. On the other hand, higher plant spacing didn't significantly increase the marketable yield per hectare. These results are in correspondence with

the findings of Semuli (2005) who mentioned that the greatest head weight resulting from increased spacing failed to compensate for the decreased number of heads that resulted from such spacing.

As expected, the effects of population spacing were primarily noted on yield and its characteristics but less on head quality.

Our experiment indicated that plant spacing can influence levels of herbivory. We have found that the damage ratings of thrips tabaci were generally higher in the sparse plots than in the dense plots. The mean damage rating of low spacing plots was 1.85, while mean damage ratings in the high spacing plots was 1.42.

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Agrovoc descriptors: brassica oleracea; thrips (genus); thrips tabaci; damage; carbohydrates; sucrose; fructose; glucose; infestation; pest resistance

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Relationship between water-soluble carbohydrate composition of cabbage (*Brassica oleracea* L. var. *capitata*) and damage levels of onion thrips

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ABSTRACT

The impact of water-soluble carbohydrate composition in relation to damage levels of onion thrips (*Thrips tabaci* Lindeman), were studied under field conditions at the Experimental Field of the Biotechnical Faculty in Ljubljana on 7 cabbage cultivar (*Brassica oleracea* L. var. *capitata*). Onion thrips showed weak preference on cabbage heads with high amount of total carbohydrate, fructose and glucose and higher preference on heads with high concentration of sucrose. While amount of total carbohydrate, fructose and glucose were negatively correlated with damage levels ($r^2 = -0.7667$; $r^2 = -0.6947$; $r^2 = -0.8263$), sucrose amount was not. There was a strong positively relationship between sucrose amount and the level of plants infested ($r^2 = +0.7378$). The cv. 'Hinova', which had the highest amount of total carbohydrate, showed itself to be the most resistant to the onion thrips.

Key words: cabbage, *Brassica oleracea*, water-soluble carbohydrate, onion thrips, damage

IZVLEČEK

VPLIV VODOTOPNIH OGLJIKOVIH HIDRATOV V ZELJU (*Brassica oleracea* L. var. *capitata*) NA POŠKODBE, KI JIH POVZROČA TOBAKOV RESAR

Vpliv sestave vodotopnih ogljikovih hidratov na poškodbe, ki jih povzročata tobakov resar (*Thrips tabaci* Lindeman), smo proučevali na 7 kultivarjih zelja (*Brassica oleracea* L. var. *capitata*), vzgojenih na Laboratorijskem polju Biotehniške fakultete v Ljubljani. Tobakov resar je pokazal majhno preferenco do zeljnih glav, ki imajo visoko vsebnost skupnih ogljikovih hidratov, fruktoze in glukoze, in veliko preferenco do glav z visoko vsebnostjo saharoze. Medtem ko je vsebnost skupnih ogljikovih hidratov, fruktoze in glukoze negativno korelirala z indeksom poškodb ($r^2 = -0,7667$; $r^2 = -0,6947$; $r^2 = -0,8263$), pa to ni veljalo za saharozo. Med koncentracijo saharoze in poškodovanimi listi je bila namreč ugotovljena močno značilna

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pozitivna povezava ($r^2 = +0,7378$). Cv. 'Hinova', ki je vseboval največji delež skupnih ogljikovih hidratov, se je pokazal kot najbolj odporen kultivar na napad tobakovega resarja.

Ključne besede: zelje, *Brassica oleracea*, vodotopni ogljikovi hidrati, tobakov resar, poškodbe

INTRODUCTION

The onion thrips, *Thrips tabaci* Lindeman, is polyphagous pest which has been recorded from 29 plant families but is particularly damaging to Brassicaceae, Liliaceae and Solanaceae (Penzes et al., 1996; Theunissen and Schelling, 1998; Richter et al., 1999). *T. tabaci* has been distributed worldwide by the trade in plants and the plant products and can cause significant economic losses in countries where is established (Weber et al., 1999). Onion thrips feed by scraping the surface of plant cells and sucking out the cell contents (Commegys and Schmitt, 1965). Destruction of the epidermal cells is accompanied by whitening and scabbing of the outer surface. The resulting damage is usually measured as an overall reduction in head size and weight where cabbages are produced. The thrip's rasping of the leaves creates an injury spot which enables various plant pathogens to gain entry, thus increasing disease problems. In addition, thrips vector plant pathogens on their mouth parts from one plant to another (Chisolm and Lewis, 1984).

In the last 25 years in Europe, problems resulting from damage caused by the onion thrips on cabbage have been found in many European countries (Penzes et al., 1996; Giessmann, 1988; Kahrer, 1992; Herold and Stengel, 1993; Legutowska, 1997; Theunissen et al., 1992; Shatilova, 1991; Ellis et al., 1994). In the last decade, the onion thrips has also become a significant pest to cabbage in Slovenia (Trdan et al., 2004).

In spite of their pestiferous status in some cabbage fields, little is known about thrips biology in this crop and the relationship between their density and cabbage yield. Therefore, thrips controls often consist of automatic preventive chemical treatments early in the season. Generally, chemical control of thrips is difficult because of their wide geographic distribution, high reproductive and dispersal rates, and wide host range (German et al., 1992). In Slovenia growers normally spray insecticide 2 to 3 times per season for control (Trdan and Žnidarčič, 2004). Edelson et al. (1989) reported that one or more thrips per plant reduced yield enough to justify the expense of insecticide treatment. Insecticide application also negatively affects populations of natural enemies, and thus may induce secondary pest outbreaks and resurgence of target pests (Hardin et al., 1995).

During the last few decades, sustainable vegetable production has been attempted to minimize the increasing problems with the use of insecticides. In order to support sustainable vegetable production, it is important to develop alternative methods of pest control. This can be achieved through the use of cultural practices such as manipulation of plant density, interplanting, and host plant resistance as well as the use of other control options. Host plant resistance and biological control are central components of any viable sustainable program.

Host plant resistance in cabbage to onion thrips is important area of research for several reasons. The growing concern and regulation of pesticides in the environment is an importance issue (Hamilton and Pike, 1997). Advantages of host plant resistance include reduced expense to the growers (Stoner, 1970). Insects are becoming resistant to pesticides, which creates more problems for control (Debach and Rosen, 1991). Thrips hide between the leaves making it very difficult to reach them with pesticide (Metcalf and Metcalf, 1993).

In general, disturbances in host biochemical composition associated with thrips damage are still poorly understood. Our research has been oriented towards the study of relationship between the onion thrips damage and the biochemical composition of the cabbage plants, particularly soluble carbohydrates.

MATERIAL AND METHODS

Host plant preference trials in the field were conducted at the Experimental Field (46° 04' N, 14° 31' W, 300 m above sea level) of the Biotechnical Faculty in Ljubljana, Slovenia. Research was performed during the 2006 growing. The experimental plots consisted of a raised bed 10 m long with three rows per bed. The experiment were arranged in a randomized complete block design with seven cabbage cultivars ('Vestri', 'Delphi', 'Destiny', 'Hinova', 'Kranjsko okroglo', 'Varaždinsko' and 'Tucana').

Cabbage cultivars were planted in the greenhouse for about 6 weeks prior to transplanting into the field. Seedlings were transplanted by hand into the field in the late April in rows 0.4 m apart with 0.3 m between plants. The plants were grown under standard cultural practices except that no insecticide was used. Standard cultural practices including drip fertigation were employed and two drip lines were laid per bed. Droppers within the line were 30 cm apart. Water was supplied by a combination of drip irrigation and natural rainfall, according to evapotranspiration data.

Plant material was harvested at commercial maturity stage. Ten plants in each plot were randomly selected and recorded for damage incidence. The amount of leaf area damaged caused by onion thrips feeding was averaged over on the first 15 outer leaves of the head. Ratings of thrips feeding symptoms on the head's leaves were made using an index scale of 6 grades: 0 (non-damaged leaf), 6 leaf totally covered by verrucae) (Stoner and Shelton, 1988; Trdan et al., 2005).

Water-soluble carbohydrates were extracted from powdered plant material following methodology of Nii (1997) and Šircelj et al. (2005). Carbohydrates were extracted from the lyophilised leaf powder with bidistilled water on ice. A few drops of 2% (w/v) sulfosalicylic acid were added to the homogenate. Extracts were subjected to an isocratic HPLC analysis (column Aminex HPX-87C 300 x 7.8 mm) using bidistilled water as solvent, at flow rate of 0.6 mL min⁻¹, run time 60 min, detection with RI detector. Three sugars were selected for quantification, representing two sugars categories: disaccharides (sucrose), and monosaccharides (glucose and fructose). The amount of carbohydrates was expressed in mg/100 g of dry weight of tissue (mg/100 g dwt).

All measured and derived data, were analysed by analyses of variance (ANOVA) using Statgraphics Plus for Windows 4.0 computer program. Character means were separated by least significant differences (LSD, $P < 0.05$) when sources of variation from ANOVAs were significant ($P < 0.05$). A regression analyses was used to determine the relationship between water-soluble carbohydrates amount and onion thrips damage.

RESULTS AND DISCUSSION

There were significant differences in the level of damage between the seven cultivars of cabbage (Fig. 1). The cultivars which had a high index of damaged leaves had also very low yields (data not shown). Cv. 'Hinova' was being more resistant to onion thrips than the other cultivars. The index of feeding damaged leaf area (measured for the first 15 outer leaves of the head) by cv. 'Hinova' was 1.127. On the other side, the highest damage rating was found in cv. 'Varaždinsko' (cabbage for sauerkraut) with index damaged of 2.833. Both cultivars form head between middle of July and middle of August.

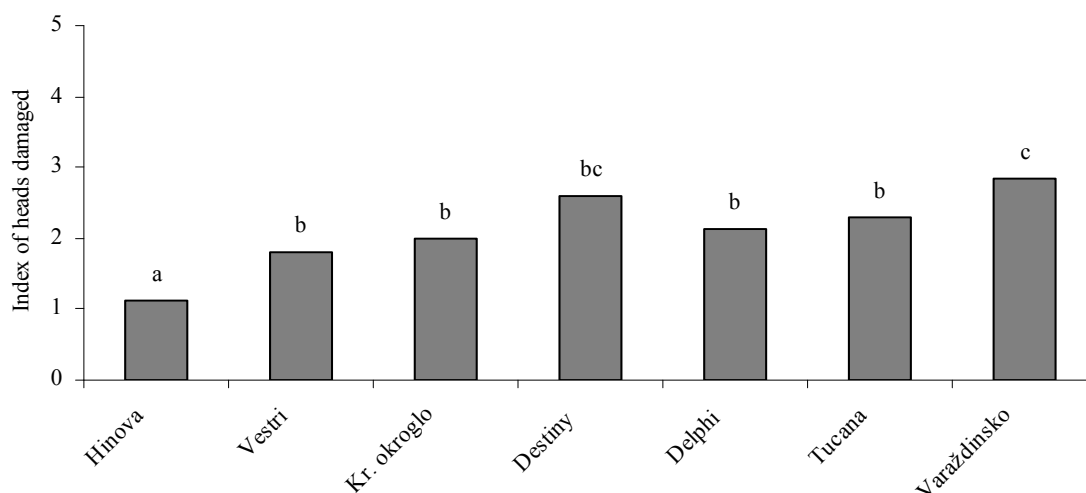


Figure 1: Mean index of heads damaged by onion thrips, *Thrips tabaci* Lindeman. Mean values followed by the same letter are not significantly different according to Duncan's Multiple Range test at $P < 0.05$.

Chemical composition of cabbage cultivars is shown in Table 1. There was a significant difference in the total water-soluble carbohydrates concentration and the concentration of each individual water-soluble carbohydrate between cabbage plants. The ratio of total carbohydrates to glucose in the leaves of cabbage examined showed that glucose accounted for more than 55% of the total sugar concentrations. Overall, higher concentration of sugars was present in the leaves of plants of cv. 'Hinova' (5389.9 mg/100 g dwt) and of cv. 'Delphi' (5256.5 mg/100 g dwt) compared to other cultivars. The greatest level of glucose was recorded in the cv. 'Hinova' (3042.1 mg/100 g dwt) and cv. 'Vestri' (2966.8 mg/100 g dwt). In contrast, cv. 'Vestri' contained the lowest level of sucrose (25.3 mg/100 g dwt). In the other cabbages the content of sucrose was relatively higher (between 30.4 mg g⁻¹ dwt in cv. 'Kranjsko okroglo' and 90.6 mg/100 g dwt in cv. 'Tucana') There were significant differences among cultivars regarding fructose amount, too. The differences established the range from 1234.2 mg/100 g dwt (cv. 'Varaždinsko') to 2265.0 mg/100 g dwt (cv. 'Delphi').

The damaged leaves, compared to healthy one, exhibited more total water-soluble carbohydrates. A relationship between the mean index of heads damaged and amount of water-soluble carbohydrates in cabbage leaves is illustrated in Fig. 2. Based on the data, the relationship is satisfactorily described by a linear function of the general form: $y = ax + b$. The mean index of heads damaged was the dependant variable (y)

and the soluble carbohydrates was the independent variable (x). A correlation coefficients of total and of the three carbohydrates indicated that there was a close correlation between the two variables. The amount of total carbohydrates, fructose and glucose in the leaves of cabbage plants correlated negatively with the damaged index ($r^2 = -0.7667$; $r^2 = -0.6947$ and $r^2 = -0.8263$). In contrast, damaged index was positively correlated to the sucrose level in the leaves ($r^2 = +0.7378$).

Table 1: Amount of water-soluble carbohydrates (mg/100 g dwt) in cabbage leaves

Cultivar	Water-soluble carbohydrates (mg/100 g dry weight tissue)			
	Glucose	Fructose	Sucrose	Total
Hinova	3042.1 c	2154.2 c	60.2 b	5389.9 d
Vestri	2966.8 c	1841.5 b	25.3 a	4833.6 c
Kranjsko okroglo	2581.4 b	1680.2 a	30.4 a	4292.0 b
Destiny	2461.8 ab	1782.4 ab	87.6 c	4331.8 b
Delphi	3062.4 c	2265.0 c	62.5 b	5256.5 d
Tucana	2341.1 a	1662.4 a	90.6 c	4094.1 b
Varaždinsko	2195.3 a	1234.2 a	86.4 c	3515.9 a

Within a column, values are not significantly different ($P < 0.05$) if followed by the same letter

Previous researches have shown that most herbivorous insects use carbohydrates as feeding stimulants (Bernays and Simpson, 1982; Blaney and Simmonds, 1988) and as important nutrients needed to synthesize body tissue and serve as energy sources (Schoonhoven et al., 1998b). Derridj et al. (1996) reported that sugars have also been shown to promote oviposition in some species. However, the data above the effect of separated sugar categories on feeding preferences of onion thrips are scarce.

The outcome of onion thrips-cabbage interactions at high carbohydrate content is the product of three main factors. Firstly, carbohydrates may directly affect thrips level damage via changes in the nutritional quality of cabbage tissue during growing season. The second factor that affected the interaction between cabbage heads and thrips was direct effects of carbohydrates on plant growth rates and therefore plant biomass available for thrips. And at last but no least, the flavour and palatability of cabbage plants is a function of relative levels of total sugars (glucose + fructose + sucrose) or reducing sugars (glucose + fructose) and presence of various aromatic constituents.

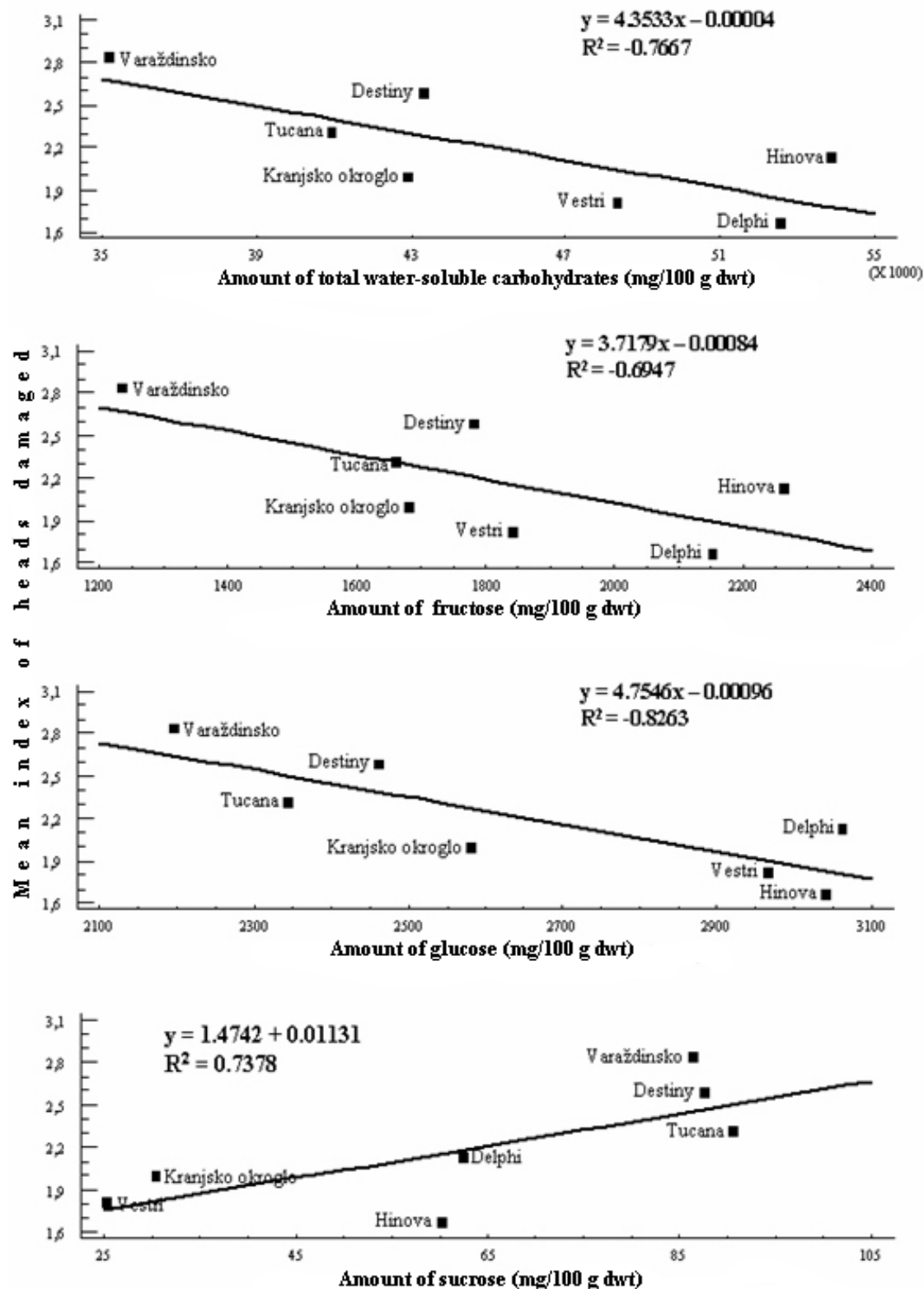


Figure 2: Regression analyses of the relationship of mean index of heads damaged to amount of water-soluble carbohydrates (mg/100 g dwt) in cabbage leaves

CONCLUSIONS

Plant-insect relationship may result in host defence, or in systemic symptoms. Our research suggests that the employment of resistant cultivars in the cultural control strategy for the long-term management of onion thrips is important. Onion thrips can discriminate among different cabbage cultivars, suggesting some kind of resistance in these cultivars that might be useful for controlling onion thrips damage in cabbage.

On the basis of our investigation of growing the cabbage the following could be concluded:

- the levels of soluble carbohydrates are dependent on cabbage cultivar;
- glucose was, on average, the major carbohydrate in the seven cultivars, followed by fructose;
- on the cv. 'Hinova' the amount of damaged leaf area, due to onion thrips feeding, was substantially lower as compared with the other cultivars;
- between carbohydrates and damaged leaf area a statistically significant correlation was observed.

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Crop management systems and endomycorrhiza effects on endive (*Cichorium endivia* L.) growth

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ABSTRACT

The goal of this research was to determine the influence of crop management systems, (organic, sustainable and conventional) and endomycorrhiza on vegetative growth of endive (*Cichorium endivia* L.) grown on fields in order to use PE mulch after lettuce crop. During 2002 and 2003 a two-factor trial with three repetitions and split-plot design was set up in Pula (Croatia). Main factor "crop management" had three levels (organic, sustainable and conventional); while the sub factor "mycorrhiza" had two levels (endive seedlings inoculated with endomycorrhizal fungus and non-inoculated seedlings). Endive was planted after lettuce harvesting on the set PE mulch in conventional and integrated management, while in organic management it was planted after tilling lettuce rests and plant mulch (fodder pea and common vetch). Fertilization (by fertirigation) and crop protection were performed according to basic principles of organic, sustainable and conventional crop management system. Neither mycorrhiza nor production systems had no significant influence on plant density, diameter and mass of heads, and marketable yields. The biggest endive head diameter had non-inoculated endive plants in first year of research, while in the second year this feature was not significant. Production system had no significant influence on head diameter. The sustainable production system had the most marketable endive heads, while the organic system had most non-marketable heads in the first research year, while in the second this feature was not significant. Mycorrhiza had no influence on this feature.

Key words: conventional production, endive (*Cichorium endivia* L.), endomycorrhiza, organic production, sustainable production

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IZVLEČEK

VPLIV NAČINA GOJENJA IN ENDOMIKORIZI NA RAST ENDIVIJE (*Cichorium endivia* L.)

Cilj raziskave je bil primerjati vplive ekološkega, integriranega in konvencionalnega gojenja endivije (*Cichorium endivia* L.) ter vpliv endomikorize na njeno rast. Endivija je bila gojena na PE zastirki kot naknadni posevek po solati. V letih 2002 in 2003 je bil dvofaktorski poskus v treh ponovitvah s split-plot zasnovu postavljen v Pulju (Hrvaška). Glavni dejavnik "način gojenja" je vseboval tri ravni (ekološki, integrirani in konvencionalni način), medtem ko je drugi dejavnik imal dve ravni (sadike endivije, inokulirane z endomikorizno glivo, in sadike brez inokuluma). Endivija je bila posajena po pobiranju solate na PE zastirko v konvencionalni in integrirani proizvodnji, medtem ko je bila v ekološki proizvodnji posajena po zaoravanju ostankov solate in rastlinske zastirke (krmni grah in navadna grašica). Gnojenje (fertiirigacija) in varstvo pred boleznimi ter škodljivci so temeljili na priporočenih metodah za ekološko integrirano in konvencionalno gojenje. Mikoriza in način gnojenja nista značilno vplivala na gostoto rastlin, premer in težo glav ter tržni pridelek. Največji premer glav so imele rastline, gojene brez inokuluma, v prvem letu raziskave, medtem ko v drugem letu med rastlinami ni bilo značilnih razlik. Način gojenja ni vplival na premer glav. Največje število tržnih glav je bilo v prvem letu doseženih v integriranem načinu, medtem ko je ekološki način dal največje število netržnih glav. Drugo leto poskusa se ta lastnost ni značilno pokazala na rastlinah. Prav tako tudi mikoriza ni vplivala na to lastnost.

Ključne besede: endivija (*Cichorium endivia* L.), ekološka pridelava, endomikoriza, konvencionalna pridelava, integrirana pridelava

INTRODUCTION

The conventional production systems from which most of vegetables originate is produced by high levels of chemization on specialized farms with high production and high inputs of materials and costs by which high yields are obtained and costs are lower per unit of production (Abdul-Baki, 1998; Shennan, 1992). Such production requires production procedures risky for the environment and people's health what leads to soil erosion and permanent soil degrading (Teasdale and Colacicco, 1985; Bašić, 1996). Also this type of conventional production requires use of polyethylene (PE) mulch which is used for many vegetable varieties which are grown from transplants (Abdul-Baki et al., 1992; Brown et al., 1992; Wien et al., 1993; Borošić et al., 1997; 1998; Romić and Borošić, 1998; Abdul-Baki, 1998; Ban et al., 2003; 2004.). Beside main positive influence that this mulch has in preventing weed growth, rising or reducing soil temperature (depending on colour), preventing disease spreading and finally improving yield quantity, these mulches are present as an ecological problem. On 87% of land in USA non degradable black polyethylene film has been used which causes additional costs for production (costs of purchase, setting up and removing the polyethylene film) and an ecological and environmental problem because it can be used only once and the question is how to recycle and waste it (Hemphill, 1993; Roe et al., 1994). Furthermore, in conventional production large amounts of fertilizers are used, especially nitrogenous ones. The reason is that nitrogenous fertilizers are a base for high yields but they are used several times more than the agricultural cultures need. The surpluses of nitrates are easily washed out of the soil, polluting the surface and ground waters (Romić et al., 1996; Abdul-Baki, 1998). In the same time too much nitrogenous fertilizer causes accumulation of unwanted nitrates in vegetables what affects human health (Ćustić, 1996).

Pesticides are used several times in conventional production even when there are no circumstances for disease or pest development, in order to prevent diseases and pest assuring the maximum possible yield what is unacceptable for ecology (Elliot and Mumford, 2002).

Recent research (Gaskell et al., 2000; Ban, 2001; Bulluck et al., 2002; Elliot and Mumford, 2002) showed possibilities of vegetable production in alternative systems of production (sustainable and organic), which are not so risky for the environment and create positive economic effects. Therefore the aim of our research was to show similar results of alternative systems of production (sustainable and organic) and influence of endomycorrhiza on vegetative growth and yields of endive (*Cichorium endivia* L.) as a late crop after lettuce, using the existing mulch, and comparing alternative to conventional systems of production.

MATERIAL AND METHODS

Biannual research was set on a family farm in Pula (Croatia) during 2002 and 2003. A two factor trial was set by split-plot scheme in three repetitions. The main factor "production system" had three levels (organic, sustainable and conventional); while the sub factor "mycorrhiza" had two levels (endive transplants inoculated with endomycorrhiza fungus *Glomus mossae* and non-inoculated transplants). The size of basic plot for the main factor "production system" was 45 m² (10 m x 4.5 m) contained three beds 1 m wide with 50 cm space between beds and were 10 m long, while the size of plots with factor "mycorrhiza" was 22.5 m² (5 m x 4.5 m), half of the plots of main factor.

The soil for the trial had neutral reaction (pH 7.07 in MKCl), contained 2.3% humus, 3.49 mg/100 g soil P₂O₅ and 17.95 mg/100 g soil K₂O. The trial land surface was ploughed 30 cm deep in February, each year. Before ploughing, on plots which are labelled for organic system of production, ripe organic manure was used (100 m³/ha) and ploughed in the soil. On organic production plots, after supplementary treatment, tilling, in mid March we have sown in the first year fodder pea variety 'Maksimirski', in the second year common vetch, variety 'Jaga', each 140 kg/ha. Both fodder pea and common vetch were mow down by end of May and were used as plant mulch for lettuce that forwarded endive.

In both years after mowing down the spring crop an drip irrigation system was set for organic production system, while for sustainable and conventional systems we used mineral fertilizers (1000 kg/ha NPK 7-20-30), and herbicide (trifluralin, 2 l/ha), we tilled, set drip irrigation systems and set mulch of black PE film 1.2 m wide. On such prepared plots in both years lettuce was grown (in beds) with 10.67 plants/m² on all trial plots. During vegetation in both years we used drip irrigation. Each year we used fertirigation, and basic measures of culture care depending on production system. Plant protection was done by State regulations: Rulebook on ecological production of plants and in total production of plant's products (Official Gazette, 2001). Lettuce harvesting was done in third decade of August. After lettuce harvesting on plots with sustainable and conventional production systems, lettuce leftovers were removed, while on PE mulch holes for planting endive transplants were made. Lettuce leftovers and plant mulch (fodder pea and common vetch) in organic production were tilled in the soil.

Inoculation of endive with endomycorrhiza fungus was done by mixing the fungus mycelium in the substrate for transplants growing (Novak, 1997.). Transplants of variety 'Laos', Seed Company Bejo zaden were grown in a polyethylene container with 150 holes (sowing on 22. 7. 2002 and 18. 7. 2003). The transplants in phase 5-6 true leaves were planted in three bed rows per plot (in each bed we had three rows) on 4. 9. 2002 and 28. 8. 2003 the interspaced between rows was 30 cm and inside rows 50 cm (three rows per bed – mulch film), while the distance between beds was 50 cm (4 plant/m²). For data processing we used data about the middle bed of each plots.

Before planting in planting holes on plots for sustainable and conventional production we used soil insecticide (Durban G-7.5) in amount of 15 kg/ha in sustainable and 20 kg/ha in conventional system. After planting on all plots we used molluscides against snails in dosage according to State regulations. In organic production we used mechanical protection and yellow tables to attract insects (aphids).

During vegetation we used irrigation according to vegetation needs, while fertilizer was applied by fertigation (plots with organic production had no fertigation), while treatments against diseases and pests were executed according to production system (Table 1 and 2).

Table 1: Fertilization regime in endive production systems

Fertilizer	Production system		
	Organic	Sustainable	Conventional
Soluble mineral fertilizer (NPK 19-6-20)	-	88 kg N/ha in two portions (every second week)	158 kg N/ha in four portions (every week)
Total kg N/ha	-	88	158

Endive harvesting was done at once on 8. 12. 2002 and 7. 12. 2003 on all plots and for all systems of production. After harvesting we determined the density of plants, the diameters of endive heads, average mass of endive heads yields and share of endive non-marketable yields.

Statistical analysis of the main factor, sub factor and their interaction was done by variance test (F-test). For significantly different average values of F-test between the main factor and its interaction, we tested them by Duncan's rang test for significance $p \leq 0.05$.

Table 2: Pesticide use in endive production systems

Pesticide	Production system		
	Organic	Sustainable	Conventional
<i>Limacides:</i> - Metaldehyde (Limax M)	1 x (30 kg/ha)	1 x (35 kg/ha)	1 x (40 kg/ha)
<i>Insecticides:</i> - Klorpirifos-etil (Dursban G-7.5) - Deltametrin (Rotor 1.25 EC) - Pyrethrum extract (Biotox P) - Yellow tables	- - 1 x (600 l/ha) 1.200 peace/ha	1 x (15 kg/ha) 1 x (0.06 %) - -	1 x (20 kg/ha) 2 x (0.06%) - -
<i>Fungicides:</i> - Metalaksil+mankozeb (Ridomil MZ 72 WP) - Iprodion (Rovral SC)	- -	- 1 x (0.30%)	1 x (0.30%) 2 x (0.30%)
<i>Herbicides:</i> - Glufosinat-amonij (Basta 15)	- (1 x weeding)	1 x (6 l/ha)	1 x (6 l/ha)

RESULTS AND DISCUSSION

Production systems, mycorrhiza and their interaction had no significant influence on density reduction in endive production during two years of research (Table 3). Snails

are one of the reasons for diminished plant density, because they were very active starting in September, when more rains are usual in Pula. After transplanting the plant density was diminished by sun burns because of black mulch and sun influence. However in our research these causes were not to blame as we used molluscides in all three systems of production and the temperatures of soil in organic and on PE mulch surface in conventional and sustainable production system were not a limitation factor (data not shown). Insignificant diminished plant density in all three production systems in planting phase were caused by mechanical damage in time of planting.

The diameter of endive head is genetically determined feature that can be changed by wider planting spaces, but also is a feature that determines planting interspaces. In our research plants were planted on a distance that made full development possible. In all production years, mycorrhiza and interaction of mycorrhiza and production system had no influence on endive head diameter. These results are opposite to results (Ban et al., 2003) where lettuce heads were significantly larger in sustainable production, than in organic. The reason lies in the fact that endive has better tolerance and endurance to many stresses (weaker nutrition, water stress) than lettuce, therefore the production circumstances for endive with no manure, weeds and soil compression had not showed effects on head diameters. The differences were not significant either comparing organic to “softer” conditions of sustainable and conventional production. Inoculation of endive transplants with endomycorrhizal fungus has showed no significant results. We assume that was because soil available nutrients were sufficient to head development.

Table 3: Effects of production system and mycorrhiza on the achieved plant density and endive heads diameter in harvest time

Production system	Plant density (plant/m ²)			Diameter of heads (cm)		
	M ¹	BM ²	PSP ³	M	BM	PSP
	2002					
Organic	3.73 a	3.91 a	3.82 a	39.73 a	41.93 a	40.83 a
Sustainable	3.82 a	3.73 a	3.78 a	41.65 a	41.68 a	41.67 a
Conventional	3.73 a	3.47 a	3.60 a	40.07 a	40.50 a	40.29 a
Mean myc.	3.76 n.s.	3.70 n.s.		40.48 n.s.	41.37 n.s.	
2003						
Organic	3.87 a	3.96 a	3.92 a	41.90 a	41.25 a	41.58 a
Sustainable	3.91 a	3.87 a	3.89 a	39.70 a	39.62 a	39.66 a
Conventional	3.96 a	3.91 a	3.94 a	41.82 a	41.55 a	41.69 a
Mean myc.	3.91 n.s.	3.91 n.s.		41.14 n.s.	40.81 n.s.	

¹Mycorrhiza; ²Without mycorrhiza; ³Production system

The head mass was positively correlated to head diameter, larger diameters means in order bigger mass under the condition that endive is harvested in full technological ripeness, respectively when the inner parts have characteristic light yellow colour. Because of these reasons, in both years, endomycorrhiza and production systems had

no influence on endive head mass (Table 4). These findings are contrary to the findings of Ban et al. (2003) in lettuce research. The reason is, as we mentioned before, that endive has higher tolerance than lettuce. Interaction of production system and mycorrhiza was significant only in the second year of research, when the highest endive head mass was achieved in sustainable production system on non-inoculated transplants. This interaction was significant probably because pedological-climatic conditions were favourable for this interaction comparing to other interactions.

Table 4: Production system and mycorrhiza effects on average endive marketable yield and on proportions of non-marketable heads in yield

Production system	Marketable yield						Non-marketable yield		
	(g/head)			(t/ha)			(% heads)		
	M ¹	BM ²	PSP ³	M	BM	PSP	M	BM	PSP
	2002								
Organic	507 a	630 a	569 a	16.3 a	22.7 a	19.49 a	6.1 a	3.4 a	4.7 a
Sustainable	565 a	633 a	599 a	19.5 a	22.4 a	20.98 a	2.2 a	1.2 b	1.7 b
Convent.	491 a	474 a	483 a	17.1 a	15.0 a	16.05 a	3.1 a	2.5 a	2.8 ab
Mean myc.	521 n.s.	579 n.s.		17.6 n.s.	20.0 n.s.		3.8 n.s.	2.3 n.s.	
	2003								
Organic	653 ab	570 b	612 a	22.8 a	20.3 a	21.58 a	1.4 a	3.7 a	2.5 a
Sustainable	550 b	697 b	624 a	19.6 a	20.4 a	20.02 a	1.7 a	4.4 a	3.1 a
Convent.	570 b	557 b	564 a	20.9 a	20.6 a	20.80 a	1.8 a	0.5 a	1.2 a
Mean myc.	591 n.s.	608 n.s.		21.1 n.s.	20.4 n.s.		1.6 n.s.	2.8 n.s.	

¹Mycorrhiza; ²Without mycorrhiza; ³Production system

The main purpose of agriculture in general is to achieve highest possible yields with less possible costs, in this way the final product costs less and the profits are higher (Abdul-Baki, 1998). Therefore endive yields per unit area are very important. In our research endive yields were tested through several factors. The results showed that production system and mycorrhiza and both their interaction had no significant influence on yields. Based on these evident we can conclude that available amounts of nutrients (applied by manure) which were differently applied in production systems (sustainable and conventional) and not used in organic (only plant mulch of hairy vetch and fodder pea was tilled in) had no effects on increased yields. Different pest treatment also had no effects on endive yields. Endomycorrhiza also had no effects on endive yields although the nutrition status was low (88 N kg/ha in sustainable system, and no manure used in organic system). Concerning other authors (Osvald et al., 2002) manure should be applied in larger doses (150 kg N/ha, 50 kg P₂O₅/ha, 250 kg K₂O/ha). In sustainable production basic soil ploughing and basic fertilization was not

possible because of PE mulch laid down for preceding crops. Therefore less additional fertilizers were used. Fertilizer was used two times in sustainable production and four times in conventional production system, in the first month and half of vegetation. We could manure forwards but that would cause no effects on yields and would cause increase on nitrates in endive leaves (at the time the temperatures were lower and days shorter what causes nitrates accumulation). Similar nitrate accumulation in head chicory was found by Čustić (1996). All three systems of production produced about 20 tons/ha although the possible yield could have been higher, up to 40 tons/ha, which had been achieved in endive production (Osvald et al. 2002). The reasons that yields were not at the maximum lie not in manure management, because in the organic system we used no manure, still the yields were the same as the yields in sustainable and conventional production, what once more approves the mentioned statement that endive is a very tolerant vegetable culture.

The only true reason for minor yields in our research should be explained through plant density (4 plants/m²), which should be at least double (10-15 plants/m²) according to research findings of Tesi (1990) and Osvald et al. (2002). Therefore, if got yield, that was amounting 20 t/ha, multiply with double number of plants, get of a final during 40 t/ha yield, which in own parts list also Tesi (1990) and Osvald et al. (2002).

First production year had consequences on shares of non-marketable production (Table 4). Organic production had 3%, respectively 2% more non-marketable endive mass from total production comparing to sustainable and conventional production. However these differences were not proved in the second research year. Reasons were climatic conditions which in the first year were favourable for disease development. Just before harvesting in organic production endive was attacked by *Botrytis cinerea* therefore a number of plants were damaged and not suitable for market sales. Inoculation with mycorrhiza and the interaction of production systems and mycorrhiza had no effects and shares of non-marketable yields. Significant differences in research of lettuce were also not proved (Ban et al., 2003). Therefore once more we proved that endive is a very tolerant vegetable culture with few requests in production.

CONCLUSIONS

Through several field researches it is determined that endive can be successfully grown in organic production system with total omission of chemical means (mineral manure and chemically produced pesticides). Such production achieves yields which are expected in sustainable and conventional production. Because of stated findings, endive is recommended for organic production. Inoculation of transplants with the fungus *Glomus mossae* had no effect on endive production in our research.

Endive production as a succeeding crop is possible without significant yield diminishment and has positive effects on using PE mulch from the preceding culture in sustainable and conventional production systems, while in organic production it uses the plant mulch ploughed in for succeeding crops.

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Transpiration of the 'Rebula' cuttings (*Vitis vinifera* L.) grafted on three different rootstocks (*Vitis* sp.)

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ABSTRACT

The purpose of the investigation was to determine the use of water in grafted vines (*Vitis vinifera* L.) in dependence on the vine (*Vitis* sp.) rootstocks. The local vine variety 'Rebula' was grafted on three different rootstocks (*Vitis* sp.): (1) on the 'Rebula' (*Vitis vinifera* L.) vine itself, (2) on the rootstock 196/17 Castel' [(*Vitis vinifera* 'Mourvèdre' x *Vitis rupestris* '1202 Couderc') x *Vitis riparia* 'Gloire'] and (3) on the rootstock 'Börner' (*Vitis riparia* x *Vitis cinerea*). Daily transpiration was calculated as a difference in the pot mass between the day of measurement and the previous day. Daily differences in the use of water were compared with leaf area and meteorological data (temperature, duration of sun light). Stem water potential (SWP) and, at the end of the trial, leaf area and mass of individual parts of plant (roots, shoot) were determined using destructive method. The dynamics of water used per leaf area unit showed the trend of reduction and the response to the changing meteorological conditions. No statistically significant differences in SWP, leaf area and water used were noticed between rootstocks, however, they existed in the final mass of shoot and roots.

Key words: grapevine, cuttings, transpiration, rootstock

IZVLEČEK

TRANSPIRACIJA CEPLJENK VINSKE TRTE (*Vitis vinifera* L.) 'REBULA' CEPLJENE NA TRI RAZLIČNE PODLAGE (*Vitis* sp.)

Namen raziskave je bil določiti porabo vode pri enoletnih cepljenkah vinske trte (*Vitis vinifera* L.) v odvisnosti od podlage vinske trte (*Vitis* sp.). Lokalno vinsko sorto 'Rebula' smo cepili na tri različne podlage: (1) na samo sebe, 'Rebula' (*Vitis vinifera* L.), (2) podlago '196/17 Castel' [(*Vitis vinifera* 'Mourvèdre' x *Vitis rupestris* '1202 Couderc') x *Vitis riparia* 'Gloire'] in (3) podlago 'Börner' (*Vitis riparia* x *Vitis cinerea*). Dnevna transpiracija je bila izračunana kot razlika v masi lonca z rastlino, med dnevom merjenja in predhodnim dnevom. Dnevne razlike v porabi vode smo primerjali z listno površino in meteorološkimi podatki (temperatura, sončno obsevanje). Določili smo vodni potencial stebela (SWP), na koncu poskusa pa, z destruktivno metodo, listno površino in maso posameznih delov rastlin (korenine, poganjek). Dinamika

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porabe vode na enoto listne površine je pokazala odziv na spremenljive meteorološke razmere in trend zniževanja. Med podlagami nismo opazili statistično značilnih razlik v vodnem potencialu stebela, listni površini in porabi vode, statistično značilne razlike med podlagami so obstajale le v končni masi poganjka in korenin.

Ključne besede: vinska trte, cepljenke, transpiracija, podlage

INTRODUCTION

The water use of grapevines has been reported in the literature, either for mature vines (Gómez-del-Campo *et al.*, 1997; Trambouze and Woltz, 2001) or young plants (Myburgh *et al.*, 1996; Williams *et al.*, 2003). As to the water requirement of 1-year-old grapevine cuttings there is a lack of knowledge, but this information may be useful in nurseries to optimise water. Furthermore, when an experimental plot with grapevine cuttings has to be set up, accurate irrigation planning is necessary for optimal cultivation.

The aim of this research was to estimate the amount of transpiration in relation with leaf area development in young grafted grapevines. At the same time, the influence of different rootstocks on this parameter was investigated, because rootstocks may affect vigour, water relations, mineral nutrition, yield and quality (Padget-Johnson *et al.*, 2000).

MATERIAL AND METHODS

The old local grapevine variety 'Rebula' (*Vitis vinifera* L.), synonym 'Ribolla gialla', was grafted to: (1) itself, i.e. grafted to 'Rebula' (*Vitis vinifera* L.), (2) rootstock '196/17 Castel' [(*Vitis vinifera* 'Mourvèdre' x *Vitis rupestris* '1202 Couderc') x *Vitis riparia* 'Gloire'] and (3) rootstock 'Börner' (*Vitis riparia* x *Vitis cinerea*). Each grafting combination was represented by three replicates (plants). During January 2002 the grafting was performed using omega graft technique, and grafts were planted in a mist bench for two months in order to have a good rooting and growth. On 9th of April 2002 the plants were transferred in 2 l pots filled with Agriperlite BPB Vic and Goldhumus Pflanzerde (3/1, v/v), and they were grown in a greenhouse at the "Antonio Servadei" experimental farm of the University of Udine, North-Eastern Italy. Evaporation from the substrate surface of the pot was avoided covering the top with aluminium foil. Percolation was negligible. The daily transpiration rate was calculated as pot weight difference between two following days. Daily differences of pot were related to the leaf area, to obtain the daily transpiration per unit of leaf area (T_a).

At the beginning of this trial, the amount of water supplied was 0.15 l pot⁻¹ d⁻¹: this level was established based on xylem-sap flow values in mature grapevines (3-4 l H₂O m⁻² leaf area d⁻¹; Sivilotti *et al.*, 2005), and considering the initial leaf area per plant of about 0.05 m². Because of rapid leaf area growth, water supply was increased up to 0.20 l pot⁻¹ d⁻¹ (1st of June 2002) and to 0.30 l pot⁻¹ d⁻¹ (14th of June 2002). The pots were daily watered and weighted at 02.00 p.m.

Leaf area of each plant was measured non-destructively: length (Lg) and width (Wd) of every leaf was converted to leaf area (y) with a previously obtained calibration function ($y = 0.6742x + 0.0144$, $R^2 = 0.98$, where $x = Lg \cdot Wd$). The calibration equation was obtained by measuring the length the width and the area of 30 leaves of 'Rebula' thus calculating the relationship. The plants were finally destroyed and leaf area measured with leaf area meter Li-Cor 3100

(Li-Cor Inc., Lincoln, NE, USA). Furthermore they were separated into roots, shoots and leaves and dried in a stove (100°C overnight), recording fresh and dry weights.

Stem water potential (Choné *et al.* 2001) was measured by a Scholander pressure chamber (P.M.S., Corvallis, OR, USA). One leaf for each plant was sampled for each thesis.

Meteorological data: temperature and solar radiation were obtained from the meteorological station of the Experimental farm "Antonio Servadei" of the University of Udine.

Statistical analysis: ANOVA together with Student-Newmann-Keuls test ($P < 0,05$) were used to ascertain differences among means (CoStat, CoHort software, Monterey, CA, USA).

RESULTS AND DISCUSSION

Leaf area

At the beginning of the growth period (20th of May 2002) leaf area ranged between 0.009 m² for plants grafted on 'Rebula' and 0.013 m² for plants on '196/17 Castel'; in the following period leaf area showed a rapid increase. On 29th June 2002, values about 0.24 m² were measured and leaf area development showed a decrease of growth rate.

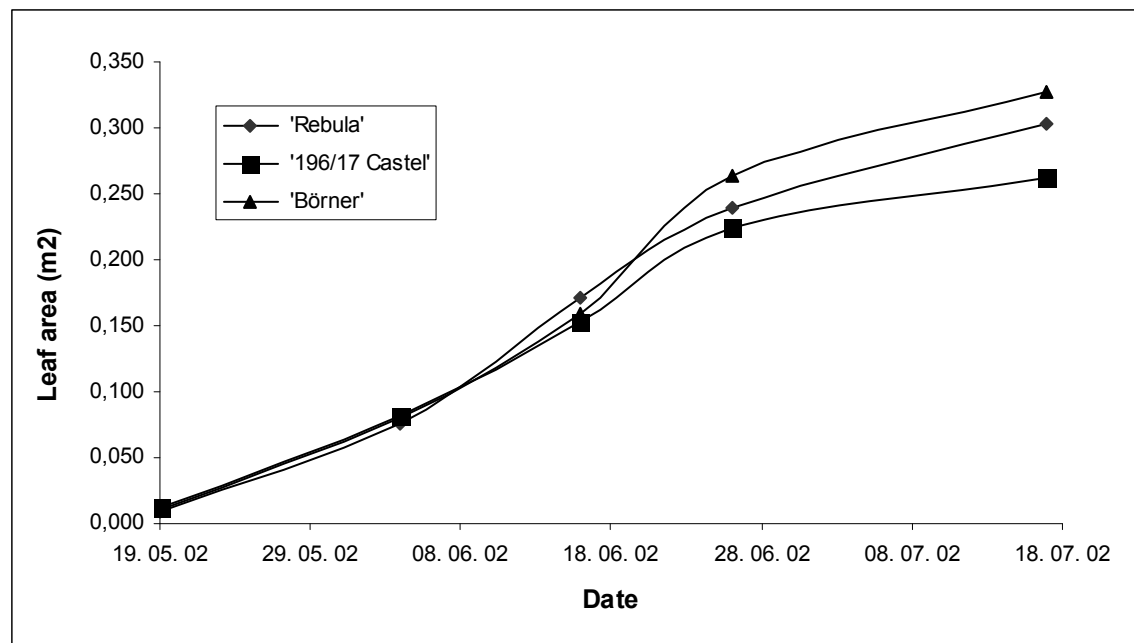


Figure 1. The leaf area of 'Rebula' grafted on three different rootstocks in different dates.

Transpiration

T_a data on 11th of June 2002 ranged between 2.24 L m⁻² leaf area d⁻¹ in 'Börner' and 2.65 L m⁻² leaf area d⁻¹ in 'Rebula'. From this moment on, T_a showed a decrease (figure 2), and no difference among rootstocks was observed. Some reductions of T_a according with meteorological changes, i.e. a decrease in temperature and radiation were observed (figure 2) particularly on 27th of June and 01st of July 2002. During

June and July the water consumption of a grafted grapevine with a leaf area of about 0.30 m^2 was quite constant with an average from 0.25 to $0.30 \text{ L H}_2\text{O d}^{-1}$ per plant.

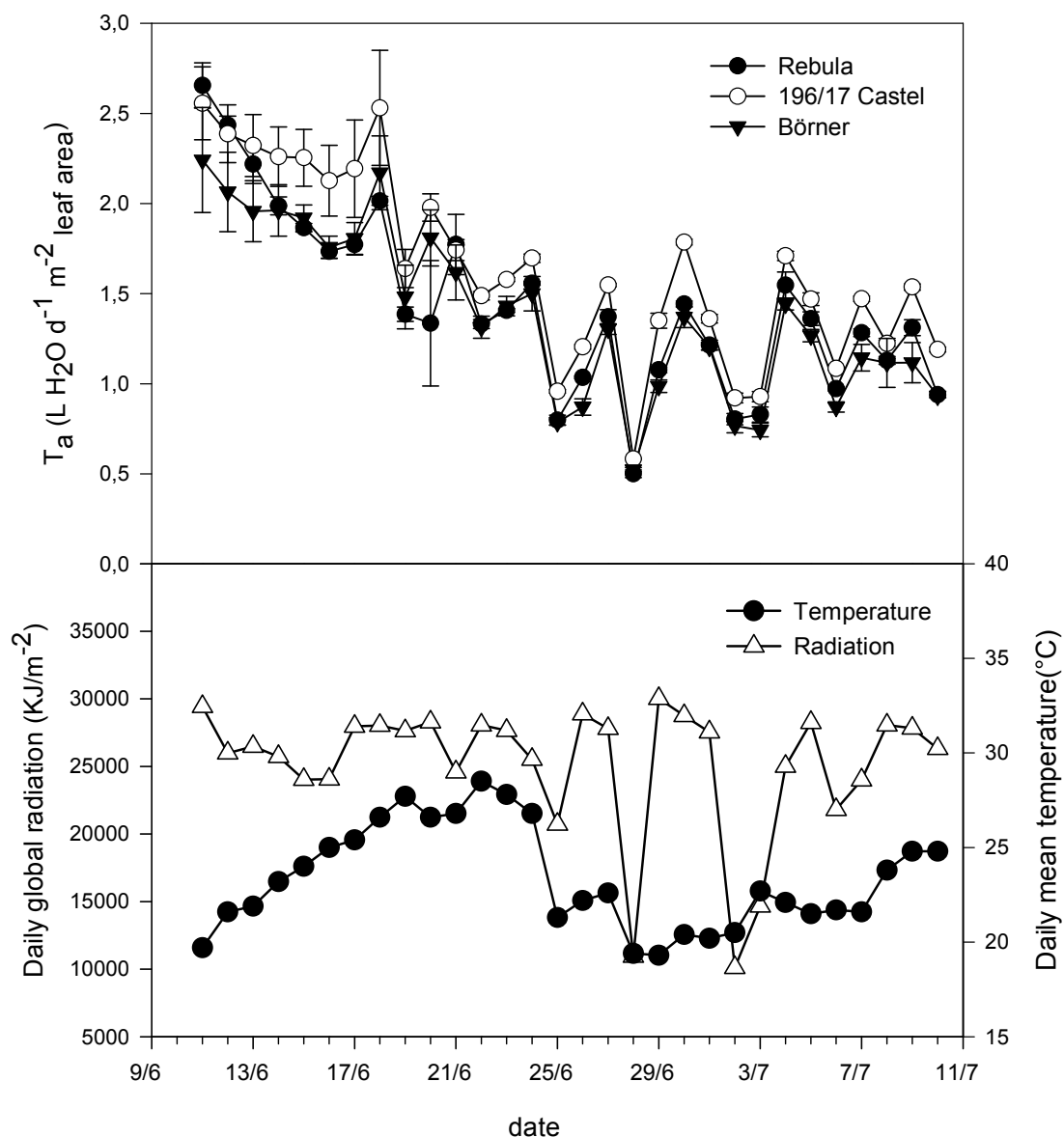


Figure 2. Young grapevine grafted cutting transpiration as affected by rootstock and meteorological changes.

Stem water potential

On 14th of June stem water potential was measured: plants on 'Rebula' showed a value of -0.53 MPa , while on '196/17 Castel' and 'Börner' -0.54 MPa without significant differences. Stem water potential revealed no water stress in the plants.

Dry matter partitioning

Dry matter partitioning revealed that 'Börner' rootstock reported the highest values of total leaf area, and the lowest value of root weight. On the opposite, '196/17 Castel'

rootstock reported the lowest values of leaf area, together with a high root weight. 'Rebula' showed mean values of all dry matter partitioning. Leaf weight did not show any difference, as well as dry matter data.

Table1: Dry matter partitioning of grapevine cuttings grafted on different rootstocks.

Rootstock	leaf area (cm ²)	leaf weight (g FW)	root weight (g FW)	root/shoot ratio
'Rebula'	3023.3 ab ^a	47.4 a	82.1 a	0.93 a
'196/17 Cl'	2617.5 b	43.6 a	81.8 a	1.03 a
'Börner'	3266.1 a	51.2 a	59.3 b	0.62 b

^a values with the same letter are not significantly different for P≤0.05.

CONCLUSIONS

The amount of transpiration was found in agreement with Trambouze and Woltz, 2001 and with Williams *et al.*, 2003 and no differences between rootstocks were assessed. The water consumption data of young grafted grapevines may be useful for irrigation scheduling in nurseries.

ACKNOWLEDGMENT

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Agrovoc descriptors: prunus avium; boron; foliar application; fertilizer application; flowers; buds; fruits; fertilization; plant nutrition

Agris category code: F61, F04

COBISS code 1.01

Effect of late season boron spray on boron accumulation and fruit set of 'Summit' and 'Hedelfinger' sweet cherry (*Prunus avium* L.)

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ABSTRACT

The influence of late-season boron (B) application on the bud B concentration and fruit set was studied in sweet cherry (*Prunus avium* L.). The experiment was carried out on 5-year-old 'Summit' and 'Hedelfinger' trees on rootstock Gisela 5 in Fruit Growing centre Gačnik. Trees were sprayed with B (1% Bortrack) or water (control). Differences in B concentration were measured between cultivars, the highest content in 'Hedelfinger' buds. Boron application resulted in increased B concentration in flower buds. Fruit set was influenced with cultivar, boron application and micro location. Fruit set was statistically higher in 'Hedelfinger' than in 'Summit' trees. The results showed that B fertilization had no effect on fruit set of 'Summit' despite increased concentration of boron by 94.8% in dormant flower buds. Foliar boron spraying of 'Hedelfinger' was effective in increasing B concentration (by 157.2%) and fruit set on half trees.

Key words: sweet cherry, *Prunus avium*, Gisela 5, foliar nutrition, boron, flower buds, fruit set.

IZVLEČEK

VPLIV FOLIARNO DODANEGA BORA V JESENI NA VSEBNOST BORA IN DELEŽ OPLODITVE PRI SORTAH 'SUMMIT' IN 'HEDELINGER' (*Prunus avium* L.)

Vpliv foliarno dodanega bora (B) v jeseni na koncentracijo bora v brstih in na delež oploditve smo proučevali pri češnji (*Prunus avium* L.). Poskus smo izvedli na 5 let starih drevesih sort 'Summit' in 'Hedelfinger' na podlagi Gisela 5 v Sadjarskem centru Gačnik. Drevesa smo poškropili z borom (1 % Bortrack) ali z vodo (kontrola). Ugotovili smo značilne razlike v koncentraciji bora med proučevanima sortama, večje koncentracije pri sorti 'Hedelfinger'. Foliarna prehrana z B je vplivala na večjo koncentracijo bora v brstih obeh sort. Ugotovili smo, da so na delež oploditve vplivali sorta, aplikacija z borom in mikro lokacija. Delež oploditve je bil pri sorti 'Hedelfinger' značilno večji kot pri sorti 'Summit'. Foliarna prehrana z borom pri sorti 'Summit' ni vplivala na delež oploditve, čeprav se je koncentracija bora v brstih po aplikaciji povečala za 94,8%. Pri sorti 'Hedelfinger' se je po škropljenju z borom

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koncentracija bora v brstih povečala za 157,2%, delež oploditve pa je bil večji le na polovici poskusnih dreves.

Ključne besede: češnja, *Prunus avium*, Gisela 5, foliarna prehrana, bor, cvetni brsti, delež oploditve.

1 INTRODUCTION

Flower clusters have a high demand for boron (B) during blossoming if fruit set is to be fully effective (Hanson and Proebsting, 1996). Application of B sprays is often used to ensure that sufficient amounts of B are available for flower fertilization, fruit set, and early fruitlet development (Peryea, 1992; Zude *et al.*, 1998; Hanson *et al.*, 1985; Stover *et al.*, 1999; Nyomora *et al.*, 1999; Štampar *et al.*, 1999; Solar *et al.*, 2001). Flower buds are a preferential sink for B mobilization after foliar application (Sanchez and Righetti, 2005). Soil-applied B increased root zone soil solution B concentrations (Neilsen *et al.*, 2004) and remained mostly in the roots while very little was translocated in the above-ground portions of the tree at full bloom (Sanchez and Righetti, 2005). The tree response to foliar application of nutrients may be inconsistent (Weinbaum *et al.*, 1988). Boron sprays after bloom increased fruit set and yield of the apple cultivar 'Elstar' (Wojcik *et al.*, 1999). Sprays at the pink flowering stage increased flower cluster and early-season leaf B concentrations of apple 'Scarlet Gala' (Peryea *et al.*, 2003). Application of B prior to flowering increased fruit set of olive 'Manzanillo' (Perica *et al.*, 2001). Foliar applications of B before full bloom or after harvest increased fruit set and fruit yield of 'Conference' pear (Wojcik and Wojcik, 2003). Boron sprays and a soil B application did not increase fruit set and production of hazelnut (Ferran *et al.*, 1997; Silva *et al.*, 2003). Cultivars with lower tissue B concentration before B application responded more significantly to application (Hanson, 1991b; Nyomora *et al.*, 1997).

Late season foliar spraying is an effective method of supplying B to flower buds, leaves and flowers (Hanson *et al.*, 1985) in sour cherry (Hanson, 1991a, 1991b), apple (Zude *et al.*, 1998), prune (Wojcik, 1999) and influences fruit set and yield of almond (Nyomora *et al.*, 1997). In vivo pollen germination and tube growth of almond were enhanced by foliarly applied B during fall (Nyomora *et al.*, 2000). Boron applied to trees in the autumn moves from the leaves into the adjacent buds, where elevated levels are maintained and expressed in flowers at anthesis (Hanson, 1991a; Thompson, 1996; Sanchez and Righetti, 2005). Apple shoot leaves retained, absorbed and exported at least three to four times as much foliage-applied boron as sweet cherry shoot leaves (Picchioni and Weinbaum, 1995).

Foliar B application often promotes fruit set and yield of different fruit species, but response to B application seems to be related to different factors, like species, cultivar, nutrient status,... The B requirements of sweet cherry and influence of B on sweet cherry fruit set are poorly understood. The purpose of the current study was to evaluate the possible differences in B concentration between sweet cherry cultivars and the influence of late-season B application on the flower bud B concentration. The aim of our experiment was also to determine if higher B concentrations influence the fruit set.

2 MATERIALS AND METHODS

The study was conducted on 5-year-old Summit/Gisela 5 and Hedelfinger/Gisela 5 sweet cherry (*Prunus avium* L.) trees, planted in a single row system (900 trees/ha) and trained as a slender spindle. Cultivars 'Kordia', 'Regina' and 'Hudson' were planted in the same orchard for good pollination between cultivars. Bees were used during blooming time to ensure good pollen distribution. Trees were without visible symptoms of boron deficiency. The soil content of boron was 0.7 ppm (Phosyn laboratories). Trial was performed in Fruit Growing centre Gačnik (Slovenia).

Foliar boron was applied 1 month prior to leaf fall (1st of October 2002) on 10 trees of each cultivar. The other 10 trees served as a control and were sprayed with water. Boron application with 1% Bortrac (0.15 ppm B, Phosyn) was settled with high pressure hand-gun sprayer. At the time of B application trees had healthy leaves – flower buds on the spurs were not free from the leaves. The experimental design was 2 randomised blocks with 20 trees of each cultivar (5 trees per plot). The position of trees of 1st block was upper part of the slope of the hill and the position of 2nd block was lower part of the slope.

Dormant buds were collected in late March 2003 for boron analyses. 20 buds from different parts of the tree were sampled (1 bud per spur). Samples (buds with bracts) were oven-dried at 45 °C without washing. The dried plant material was ground to powder. Boron was extracted with HNO₃ in Biotechnical faculty Ljubljana. Samples were analysed for B with ICP OES (Inductively Coupled Plasma – Optical Emission Spectrometer) on National Institute of Chemistry Slovenia.

Flowers and mature fruits were counted out on three comparable branches on three different parts of every tree 1 to 2 m above ground level. Fruit set was calculated from number of flowers and mature fruits on individual branches. Flowers were counted at the beginning of flowering and mature fruits at the picking time. Sweet cherry fruits were picked at commercial maturity on the basis of subjective estimation of fruit colour.

Multiway ANOVA was used for analysis the effect of factors: boron application, cultivar and micro location on boron content and fruit set. Differences between treatments were estimated with LSD test and Duncan's multiple range test ($\alpha < 0.05$). Statistical analyses were carried out using Statgraphics Plus 4.0 software (Manugistics, USA).

3 RESULTS

3.1 Climatic conditions

Climatic conditions before and during flowering are shown in Fig. 1. Figure 1 presents air temperature 2 m above the ground level in the period 6th April to 30th April 2003. In the period from April 6 to 9 spring cool spell (T_{\min} -4.8 °C) passed Slovenia which coincided with phenological stage bud bursting (first swell to side green) of sweet cherry. During full bloom the temperatures were rather low for effective pollination. After the flowering (in May) the climate was very dry, average temperatures were for 2 °C higher than long term temperatures.

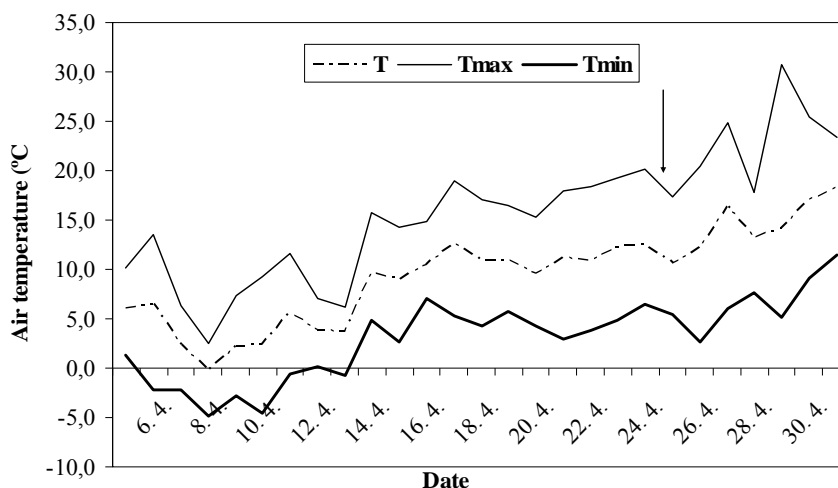


Fig. 1: The daily temperature data in the period 6th April to 30th April 2003 in Fruit growing centre Gačnik. Vertical arrow presents the phenological stage first bloom of sweet cherries.

3. 2 Boron concentrations

Differences in boron concentration in dormant flower buds were statistically significant between cultivars (Tables 1 and 2). The average boron concentration in control trees of ‘Summit’ was $127.4 \mu\text{g g}^{-1}$ dry wt and $226.2 \mu\text{g g}^{-1}$ dry wt in ‘Hedelfinger’. The single boron application resulted in higher boron content in ‘Summit’ (Table 1) and ‘Hedelfinger’ dormant buds (Table 2). Boron concentrations were similar in different blocks. Only in control ‘Summit’ trees there were higher boron concentration in Block II. Boron spray increased B concentration in ‘Summit’ buds by 94.8% and in ‘Hedelfinger’ buds by 157.2%. The average boron concentration in treated trees of ‘Summit’ was $248.2 \mu\text{g g}^{-1}$ dry wt and in ‘Hedelfinger’ $583.4 \mu\text{g g}^{-1}$ dry wt.

Table 1. Average B concentrations ($\mu\text{g g}^{-1}$ dry wt) in dormant ‘Summit’ sweet cherry flower buds. Values followed by a different letter are significantly different at $\alpha < 0.05$ by LSD test.

		Block I	Block II
Summit	Boron	267.2 ± 14.7 a	224.5 ± 20.84 a
	Control	117.6 ± 4.7 c	137.2 ± 6.1 b

Table 2. Average B concentrations ($\mu\text{g g}^{-1}$ dry wt) in dormant ‘Hedelfinger’ sweet cherry flower buds. Values followed by a different letter are significantly different at $\alpha < 0.05$ by LSD test.

		Block I	Block II
Hedelfinger	Boron	494.0 ± 120.0 a	636.8 ± 141.6 a
	Control	266.7 ± 36.9 b	197.0 ± 40.7 b

3.3 Fruit set

The number of flowers per branch was in 'Summit' and 'Hedelfinger' trees similar in all treatments. The number of 'Summit' fruits per branch was in Block I statistically higher than in Block II. Average number of 'Hedelfinger' fruits per branch in boron treated trees was statistically higher in Block I, in control trees there were no differences between blocks (data not shown).

The results show higher fruit set for 'Hedelfinger' than for 'Summit' sweet cherry. Boron application of 'Summit' sweet cherry trees resulted in similar fruit set in both treatments and both Blocks (no positive effect on fruit set). Fruit set was statistically higher only in control 'Summit' trees in Block I. Fruit set of boron treated 'Hedelfinger' sweet cherry trees was higher than of control trees only in Block I. Fruit set of 'Hedelfinger' in Block II was lower than in Block I, similar to control trees in Block I (Fig. 2).

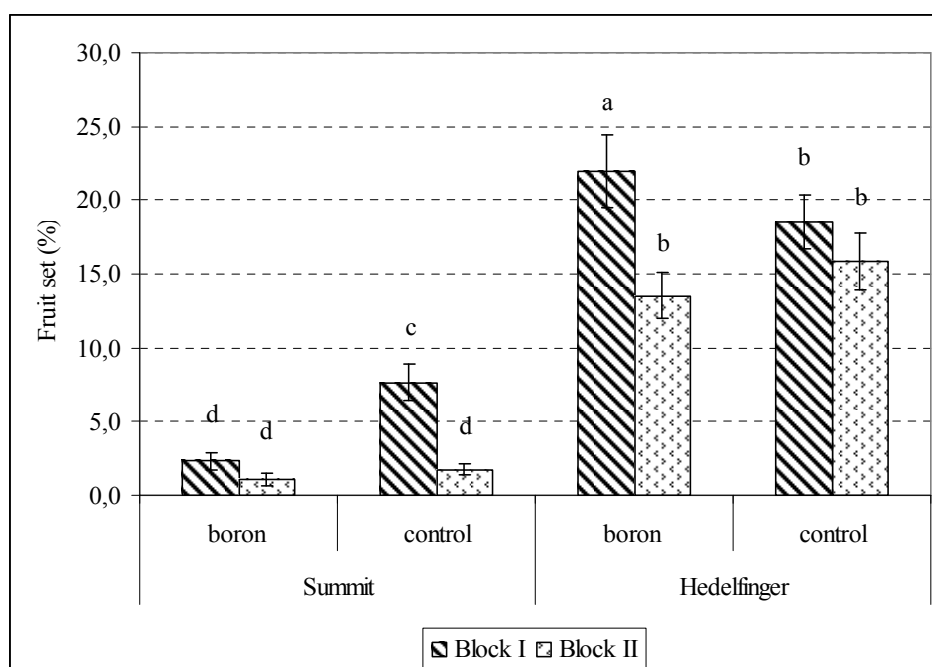


Figure 2: Fruit set (%) of 'Summit' and 'Hedelfinger' sweet cherries of different treatments and blocks. Values followed by a different letter are significantly different at $\alpha < 0.05$ by Duncan's multiple range test.

4 DISCUSSION

Single B product was used in our trial, because different B products resulted in similar flower cluster B content in apple (Peryea, 2005). The content of boron in sweet cherry dormant buds in our experiment was rather higher than content of B in sour cherry 'Montmorency' in Michigan (Hanson, 1991b). The differences in B content can be ascribed to different species and also to the rootstock used. B nutritional status of apple trees is influenced by a rootstock (Wojcik *et al.*, 2003) and can holds true also

for different sweet cherry rootstocks as well. Rootstock genotypes affect sweet cherry vegetative and generative development (Usenik *et al.*, 2006) but there are no data about the influence of rootstock on B nutritional status in sweet cherry. Analyses of boron content were made in flower buds because they can be used to predict B nutritional status of apple trees (Wojcik, 2002).

Different B concentrations were measured in 'Summit' and 'Hedelfinger' dormant flower buds. The concentration of boron in control 'Hedelfinger' trees were even higher than in 'Summit' trees after boron application. Application with boron in our trial resulted in similar increase of B like in sour cherry buds (Hanson, 1991b). Our results show that fall foliar B application is an effective method to enlarge boron concentration in sweet cherry dormant buds, like in apple (Zude *et al.*, 1998), prune (Wojcik, 1999) and sour cherry (Hanson, 1991b).

'Summit' fruit set was very low, it was less-than-optimum. Low fruit set could be influenced by several factors: young trees, fruiting habit of cultivar 'Summit' (Bargioni, 1996), climatic conditions during bud swelling (Fig. 1). T_{\min} reached -4.8 °C when sweet cherry trees were in phenological stages first swell to side green. Critical temperatures for these phases have been established for 'Bing' sweet cherry, from -11.1 to -5.8 °C (Thompson, 1996). The number of 'Summit' fruits per branch was in Block I statistically higher than in Block II which can be explained with the influence of micro location on bad pollination. On the basis of our results for 'Summit' we cannot confirm the statement that response to B application appears to be better in years when fruit set is low (Nyomora *et al.*, 1999; Perica *et al.*, 2001; Hanson in Breen, 1995; Shrestha *et al.*, 1987; Usenik and Štampar, 2002) because boron foliar application had no influence on 'Summit' fruit set. Micro location and cultivar characteristics had greater influence. Other unknown factor(s) may ultimately determine the percentage of fruits that remain on the tree at harvest (Nyomora, 1999).

Fruit set of 'Hedelfinger' sweet cherry was statistically higher than in 'Summit' sweet cherry. Fruit set of control and with B treated 'Hedelfinger' trees was sufficient for yield of good quality fruits. Boron application had positive effect on 'Hedelfinger' fruit set in Block I. Fruit set of boron treated 'Hedelfinger' sweet cherry trees was statistically higher than in control trees in Block I and higher than fruit set in both treatments in Block II. Our results in 'Hedelfinger' show that nutrition with boron can be effective (but inconsistent) also in cultivars with higher B concentrations. Nutrition with boron can be more effective in conditions where trees were supplied inadequate or in cultivars with lower tissue B concentration (Hanson, 1991b, Nyomora *et al.*, 1997).

Our results show that the tree response to foliar application of nutrients is also in sweet cherry inconsistent (Weinbaum *et al.*, 1988) and depends on different factors. Assuring a good nutritional status of the floral buds may overcome bad climatic conditions (Sanzol, 2001). Single foliar boron application is effective in increasing B concentration in flower buds, higher B concentrations however can improve fruit set in sweet cherry, so the possible positive effects can easily cover the costs. Nutrition with boron can be more useful especially when fruit set is low and can be in function of controlling tree vigour (medium productive cultivars on vigorous rootstocks). Rootstock Gisela 5, used in our experiment, is early-bearing and very productive

rootstock. There is a potential problem of excessive cropping (especially in combination with very productive cultivars) which can result in poor fruit quality and poor vegetative growth (Long, 2001). So caution is needed. Further studies of B requirements and influence of boron on sweet cherry yield are needed, especially when new technologies (cultivars, rootstocks, training systems) have been introducing.

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Effect of coir pith based cyanobacterial basal and foliar biofertilizer on *Basella rubra* L.

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ABSTRACT

The growth promoting effect of basal and foliar application of coir pith based cyanobacterial biofertilizer on *Basella rubra* L. was determined by analyzing the morphological and biochemical parameters of the control and test plants after treatment. Compared to control plants test plants showed better height, stem circumference, number of branches, number of leaves and number of flowers. Biochemical analysis of test and control plants also revealed similar increase of parameters in test sample. Thus, from our study we recommend cyanobacteria be considered as a serious contender in the field of biofertilizer.

Key words: *Oscillatoria annae*; coir pith; biofertilizer; *Basella rubra* L.; foliar spray.

IZVLEČEK

VPLIV BIOGNOJILA IZ OSTANKOV KOKOSOVIH OREHOV IN CIANOBAKTERIJ NA ZDRAVILNO ZELIŠČE *Basella rubra* L.

Raziskan je bil vpliv biognojila, dobljenega iz ostankov kokosovih orehov in predelanega s pomočjo cianobakterij na rast rastlin zdravilnega zelišča *Basella rubra* L. Avtorji so analizirali morfološke in biokemične parameter rastlin tretiranih z gnojilom in kontrolnih rastlin. S preparatom tretirane rastline so bile višje, imele so debelejšo steblo, bile so bolj razvejane in imele več listov in cvetov. S cianobakterijami obdelani organski ostanki po predelavi kokosovih orehov bi lahko bili pomembna osnova za pridobivanje biognojil.

Ključne besede: *Oscillatoria annae*; kokosovi ostanki; biognojilo; *Basella rubra* L.; foliarno gnojenje.

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1 INTRODUCTION

Cyanobacteria play a spectrum of remarkable roles in the field of energy production, biofertilizer, human food, animal feed, polysaccharides, biochemical and pharmaceuticals and in cleaning up of the environment, etc. Cyanobacteria, the oxygen evolving photosynthetic prokaryotes are found in varied aquatic and terrestrial habitats in nature as well as in association with other organisms. They show high flexibility and adapt themselves to varied environments because of their tropic independence to carbon in a number of cases. Cyanobacteria are capable of abating various kinds of pollutants and have advantages as potential biodegradation organism (Malliga and Viswajith, 2005). These organisms degrade various aromatic hydrocarbons and are useful for metal removal from polluted water. As these organisms have simple growth requirements, they could be attractive host for production of valuable organic products.

Coconut (*Cocos nucifera* L.) is cultivated in tropical countries. The fibrous mesocarp of coir is used to make ropes. The wastes of coir yarn industry get accumulated in large quantities making their disposal difficult, though it is used as soil conditioner. Biofertilizer contains all the nutrients required for the plants and helps to increase the quality of the soil with a natural microorganism environment. Foliar spray could be used in many different growing medium with excellent production results for organic farming, industrial and home application such as gardening. *Basella rubra* belonging to the family Basellaceae is herbaceous twiners, leaves alternate, simple, penninerved, margin entire, petiolate, stipulate, flowers pink in color. *B. rubra* stems and leaves are sweet, cooling, emollient, aphrodisiac, laxative, haemostatic, appetizer, sedative, diuretic and tonic. *B. rubra* has wide distribution in tropical Asia and Africa often cultivated (Matthew, 1983; Sammbamurty, 2006).

2 MATERIALS AND METHODS

2.1 Organism and culture conditions

Fresh water cyanobacteria belonging to *Oscillatoria annae* was obtained from the germplasm of National Facility for Marine Cyanobacteria, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India. The culture was maintained in BG11 medium (Rippka *et al.*, 1979) at 1500 lux at 25 ± 2 °C.

2.2 Lignocellulosic material

Coir pith was collected from coir pith industry, Tiruchirappalli, Tamilnadu, India.

2.3 Experimental condition

Coir pith was collected from coir pith industry near Tiruchirappalli, Tamilnadu, India. A shallow pit was prepared 6 m length and 1.5 m width and a rexin sheet was stretched over the pit. 100 litre of water was added to the pit. The pit was inoculated with 1 kg of *O. annae*. After 3 days coir pith was added in the ratio 1: 100. Cyanobacteria were allowed to act on the coir pith for 15 days. Coir pith with cyanobacterium was filtered, mixed with soil in 1:1 ratio and used as basal biofertilizer. The supernatant was applied as foliar spray. Totally 14 stem cuts were used for this experiment. Basal and foliar spray was applied on seven cuts which were treated as test. Seven stem cuts without any treatment act as control.

After 40 days of treatment morphological parameters like height of the plant, stem circumference, branching and number of leaves and flowers of *B. rubra* were analysed.

Biochemical parameters like sugars (Dubois *et al.*, 1956), total phenolics (Swain and Hillis, 1959) nitrate (Wolley *et al.*, 1960) chlorophyll (Arnon, 1949) carotenoid (Goodwin, 1954) and protein (Lowry *et al.*, 1951) were evaluated.

3 RESULT AND DISCUSSION

O. annae grew luxuriantly along with coir pith in field condition. During degradation the medium color changed from colorless to brown due to the release of phenolic compounds into the medium. This clearly indicated that the cyanobacterial growth was not inhibited by the presence of the lignin content in coir pith. Combined effect of basal and foliar application of coir pith based cyanobacterial biofertilizer had a positive impact on the growth of *B. rubra*. Morphological observation in control and test plant of *B. rubra* (Table–1) demonstrated increase in stem circumference (104.5 %), branching (244.36 %), number of flowers (600 %), number of leaves (108.9 %) and height of test plants (31.7 %) over control. Previous studies shows appreciable supporting results. Kumar and Mohan (1997) reported that seaweed liquid fertilizer increased the number of flowers in black gram. This increase in number of leaves was in agreement with our result showing increase in flowers in test sample. Lavanya priya (1997) and Krishna veni (1999) showed a considerable increase in the growth of rice plants with coir pith based cyanobacterial biofertilizer. Spraying of cyanobacterial extract led to increased growth and yield of black gram plant (Ravishankar, 2000). Stephenson (1974) reported a 61% increase in tomato yield with liquid seaweed extract. Zeenat and Sharma (1990) observed the effect of application cyanobacteria in combination with the chemical fertilizer diammonium phosphate on the growth on yield of tomatoes. The above mentioned results completely supported our study of combining basal and foliar application of coir pith based cyanobacterial biofertilizer.

Table 1: Comparison of morphological parameters of coir pith based cyanobacterial biofertilizer treated and untreated *B. rubra*.

S. No.	Morphological parameters	Control	Test	% of increase over control
1.	Height (cm)	16.1±0.021	21.2±0.026	31.7
2.	Stem circumference (cm)	1.55±0.013	3.17±0.018	104.5
3.	Number of branches	2.57±0.024	8.85±0.033	244.36
4.	Number of leaves	11.2±0.001	23.4±0.020	108.9
5.	Number of flowers	5±0.031	35±0.042	600

Values are the mean of three replicates ± SD.

Table 2: Comparison of biochemical parameters of coir pith based cyanobacterial biofertilizer treated and untreated *B. rubra*

S. No	Sample	Sugar (µg/ml)	Phenol (µg/ml)	Nitrate (µg/ml)	Chlorophyll <i>a</i> (µg/ml)	Carotenoid (mg/ml)	Protein (mg/ml)
1.	Control	59.3±0.023	12.6±0.107	13.7±0.01	101.0±4.728	0.07±0.01	26.0±0.035
2.	Test	78.6±0.035	16.5±0.042	15.5±0.125	144.5±8.922	0.08±0.012	78.5±0.04
3.	% of increase over control	32.5	30.9	13.1	43.0	14.2	201.9

Values are the mean of three replicates ± SD.

The water holding capacity of coir pith is enormous which was a boost to the growth of cyanobacteria. The non-nitrogen fixing cyanobacteria which enriched the phosphorus and potassium content in the soil also played a major role. This may be due to release of growth promoting substance from cyanobacteria Selvarani (1983). Combination of water holding capacity of coir pith and enrichment of soil by non-nitrogen fixing cyanobacteria resulted in enhancement of growth of test plants. This was clearly evident from the biochemical analysis of control and test plants. Biochemical analysis showed an increase in sugar (32.5 %), phenol (30.9 %), nitrate (13.1 %), protein (201.9 %), chlorophyll *a* (43.0 %) and carotenoid (14.2 %) in test plants over control. Thus from our results and earlier reports we conclude that coir pith based cyanobacterial biofertilizer could be an effective alternative or combination for chemical fertilizer.

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Selenium and plants

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ABSTRACT

Selenium is of metabolic importance in cyanobacteria and in some plants, being involved in their antioxidative processes. Selenium is widely distributed on the Earth's surface and available for plants in at least small traces. Cultivation of plants enriched with selenium could be an effective way of producing selenium rich foodstuffs and thereby increase health benefits. The essentiality of selenium to higher plants is still under debate. Selenium can increase the tolerance of plants to UV-induced oxidative stress, delay senescence, and promote the growth of ageing seedlings. Recently it has been shown that selenium has the ability to regulate the water status of plants under conditions of drought. The distribution and speciation of selenium in plants and the effect of selenium alone and in combination with some other environmental parameters is discussed.

Key words: selenium, plants

IZVLEČEK

SELEN V RASTLINAH

Selen ima pomembno vlogo pri antioksidativnih procesih cianobakterij in nekaterih rastlin. Selen je široko razširjen po zemeljski obli in na razpolago rastlinam vsaj v majhnih količinah. Gojenje rastlin, obogatenih s selenom, je učinkovit način dodajanja selena ljudem in izboljšanju zdravja. V znanstvenem svetu poteka debata, ali je selen potreben za rastline. Obstajajo pa dokazi, da selen pri rastlinah pospešuje antioksidacijsko aktivnost, zavira procese, povezane s staranjem in omili stres zaradi visoke svetlobe in tudi suše. V članku je opisana sposobnost rastlin za akumulacijo selena in vpliv selena v kombinaciji za nekaterimi drugimi okoljskimi dejavniki na rastline.

Ključni besedi: selen, rastline

1 INTRODUCTION

Selenium is a trace element that can function as an essential nutrient for humans and animals or as an environmental toxicant; the boundary between the two is narrow and

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depends on its chemical form, concentration, and other environmentally regulating variables (Fan et al., 2002; Shardendu et al., 2003). It is an important microelement, exists in small amounts in microorganisms, plants, animals and humans. Although selenium is an essential trace nutrient important to humans and most other animals as an antioxidant, toxicity occurs at high concentrations due to replacement of sulphur with selenium in amino acids resulting in incorrect folding of the protein and consequently nonfunctional proteins and enzymes.

2 BENEFICIARY EFFECT OF SELENIUM TO HUMANS

Some regions like Slovenia, suffer from a relative deficiency of selenium (Pirc and Šajn, 1997). Cultivation of plants enriched with selenium could be an effective way of producing selenium rich foodstuffs and thereby increase health benefits (Ip and Lisk, 1994; Finley et al., 2001; Lyons et al., 2005). Selenium has an important role in the prevention of atherosclerosis, specific cancers, arthritis, and altered immunological functions. The beneficial effects of selenium are dependent on the chemical form. Selenomethionine (SeMet) is known to be the most readily assimilated form of selenium (Patrick, 2004). Supplementation of human diet with selenium yeast, containing SeMet as the main chemical form, significantly reduced the occurrence of prostate cancer (Duffield-Lillico et al., 2003).

3. EFFECT OF SELENIUM ON PLANTS

The essentiality of selenium to higher plants is still under debate (Terry et al., 2000). Selenium can increase the tolerance of plants to UV-induced oxidative stress, delay senescence, and promote the growth of ageing seedlings (Xue et al., 2001; Pennanen et al., 2002). Recently it has been shown that selenium has the ability to regulate the water status of plants under conditions of drought (Kuznetsov et al., 2003). Hartikainen et al. (2000) reported about growth promoting effect of selenium in ryegrass. Senescence stress is partly counteracted with enhanced antioxidation which is associated with an increase glutathione peroxidase (GSH-Px) activity. Although some studies have evaluated the effect of hardness, temperature, pH and other parameters on selenium toxicity, sulphate has perhaps been most widely studied in relation to selenium uptake and toxicity in aquatic and terrestrial organisms (Sappington, 2002). Selenium and sulphur are nutrients with very similar chemical properties and their uptake and assimilation proceed through common pathways (Eapen in D'Souza, 2005).

4 ABILITY OF PLANTS TO ACCUMULATE SE

Se has not been classified as an essential element for plants, although its role has been considered to be beneficial in plants capable of accumulating large amounts of the element (Terry et al., 2000). Uptake and accumulation of selenium by plants is determined by the chemical form and concentration, soil factors such as pH, salinity and CaCO₃ content, the identity and concentration of competing ions, and the ability of the plant to absorb and metabolize selenium (Kabata Pendias, 2001). Actively

growing tissues usually contain the largest amounts of selenium (Kahakachchi et al., 2004). Plants usually accumulate more selenium in shoot and leaf than in root tissues (Zayed et al., 1998).

5 RESEARCH IN SLOVENIA

5.1 Methodology

On Jožef Stefan Institute, Biotechnical faculty and National Institute of Biology, the determination of selenium in plants and their species, and on the other hand the effect of selenium on plants are studied (Smrkolj and Stibilj, 2004; Breznik et al., 2005; Germ et al., 2005a,b; Smrkolj et al., 2005; Smrkolj et al., 2006a). Selenium is applied either as foliarly spraying, either by soaking the seeds in the solution of selenium. Model plants are cultivars of *Phaseolus vulgaris*, *Eruca sativa*, *Pisum sativum*, *Glicine max*, *Fagopyrum esculentum*, *F. tataricum*, *Hypericum perforatum*, *Cucurbita pepo*,...). Experiments are conducted in glass houses as well as in semi-controlled conditions and outdoors. The effect of selenium on plants is in certain cases combined with other factors, like UV-B radiation (*Fagopyrum esculentum*, *F. tataricum*, *Cucurbita pepo*) and drought (*Solanum tuberosum*).

5.1 Distribution of selenium and its species in plants

It was shown, that pea was good accumulators of selenium. The selenium content of pea seeds obtained from the untreated (UT group), once (OT) and twice (TT) foliarly treated plants was determined. The selenium content of pea seeds obtained from the untreated, once and twice foliarly treated plants was, in each case, directly proportional to the number of spraying applications. Seeds are usually a moderate source of selenium, but several studies dealing with cereal and legume seeds showed, that they are able to accumulate high amounts of selenium (Stadlober et al., 2001; Smrkolj et al., 2005; Smrkolj et al., 2006a). Higher selenium contents were found in leaves than in stems of plants grown from both OT and TT seeds, and these contents were, as for the seeds, directly proportional to the number of original foliar treatments (Smrkolj et al., 2006a). Selenomethionine was the only selenium compound found in supernatants by anion and cation exchange chromatography, comprising 49 and 67 % of the total selenium content in the OT and TT groups, respectively (Smrkolj et al., 2006a). The results of the study show that selenium enriched pea seeds are potential source of dietary selenium, on account of their ability to accumulate Se, and that this selenium is present mainly as SeMet, known to be favourable for human consumption (Smrkolj et al., 2006a).

Common (*Fagopyrum esculentum*) and tartary (*Fagopyrum tataricum*) buckwheat was treated by spraying the leaves with a water solution containing in the form of sodium selenate in the flowering period. The selenium content in all parts of plant was found to be less than 200 ng g⁻¹ in non-treated and in the range 2700–4650 ng g⁻¹ in selenium treated buckwheat. Exposure to UV-B radiation lead to higher selenium accumulation in flowers of both selenium enriched cultivars. In flowers and leaves, on average 11% of the selenium content was soluble and in the form of Se(VI), representing between 0.6% (flowers) and 3% (leaves) of the selenium content. In

seeds 93% of the selenium content was found in the extracts and the main selenium species was SeMet with $93 \pm 5\%$ relative to the selenium content (Smrkolj et al., 2005).

Determination of selenium compounds was performed in chicory (*Cichorium intybus*) leaves from plants which were cultivated aeroponically with elevated concentrations of Na_2SeO_4 for different periods. Selenium accumulated efficiently in chicory leaves; up to $480 \mu\text{g/g}$ after 41 days of exposure, mostly (64%) as Se^{VI} , i.e. in the form of selenium added. Beside inorganic Se, they also found SeMet (4.2–8.4%) and SeMeSeCys (<DL–0.7%) (Mazej et al., 2006).

Data of content of selenium and its species in different plants are shown in Table 1.

Table 1. Literature data of content of selenium and its species in leaves of different plants.

Plant	Se addition	Total Se in leaves ($\mu\text{g/g}$ dry weight)	SeMeSeCys (%) ^a	Literature
Chicory (leaves) (<i>Cichorium intybus</i> L.)	Na_2SeO_4 7 mg/L 41 days aeroponically	167-480	0.5-1.7	Mazej et al., 2007
Lamb's lettuce (leaves) (<i>Valerianella locusta</i> L.)		455	3.0	
Dandelion (leaves) (<i>Taraxacum officinale</i> Waggers)		49	2.0	
Parsley (leaves) (<i>Petroselinum crispum</i> Mill.)		290	4.4	
Bean (seeds) (<i>Phaseolus vulgaris</i> L.)	Na_2SeO_4 10 mg/L 10 days	2	30	Smrkolj, 2006b

^a - % SeMeSeCys relative to total Se in sample

5.2 The response of plants to selenium and with combination with other environmental parameters

Physiological parameters were measured in control plants and in plants obtained from selenium treated seeds in *Eruca sativa* and in foliarly treated *Fagopyrum esculentum* and *F. tataricum*. The potential and effective quantum yields of photosystem II were unaffected by selenium in *Eruca sativa*, *Fagopyrum esculentum* and *F. tataricum* (Germ and Osvald, 2005; Breznik et al., 2005a). The respiratory potential of *Eruca sativa*, measured by electron transport system (ETS) activity, significantly increased in plants, grown from selenium treated seeds (Germ and Osvald, 2005). The similar results were given in the study on young plants of *P. sativum*; ETS activity was highest in young pea leaves with the highest selenium content (Smrkolj et al., 2006a). Selenium induced higher respiratory potential in the leaves of foliarly treated selenium in *S. tuberosum* cv. Desiree, (unpublished data). Higher ETS activity reflected increased GSH-Px activity in mitochondria (Smrkolj et al., 2006a). It was

evidenced (Xue and Hartikainen, 2000; Hartikainen et al., 2000; Xue et al., 2001) that selenium exposure increased GSH-Px activity in ryegrass and lettuce. However, selenium did not affect respiratory potential in *F. esculentum* and *F. tataricum* (Breznik et al., 2005a), foliarly treated with selenium.

5.3 The combined effect of selenium with other environmental parameters

The addition of selenium mitigated the negative effect of UV-B radiation on effective quantum yield of PSII in both buckwheat species. The protective effect of selenium is important because it improves light harvesting and through this the availability of energy for plants (Breznik et al., 2005a). An ameliorative effect of Se, with regard to mass of the tubers, was observed in drought exposed in *S. tuberosum* cv. Desiree (unpublished data). Recently it has been shown that selenium has the ability to regulate the water status of plants under conditions of drought; selenium causes enhanced water retention in wheat tissue (Kuznetsov et al., 2003). The influence of ambient and filtered solar UV-B radiation and of selenium treatment was determined in pumpkins, *Cucurbita pepo* L. It was shown, that selenium increased yield under ambient radiation conditions (Germ et al., 2005).

Enhanced UV-B radiation negatively affected the aboveground biomass and number of seeds in foliarly treated *F. esculentum* with selenium. The interactions between selenium and UV-B radiation to aboveground biomass, number of nodes and number of seeds were obtained (Breznik et al., 2005b)

The aim of our future work is the continuation research regarding of the ability of plants to accumulate selenium, determination of selenium species in plants, and the effect of selenium on physiological and morphological characteristics of plants. We will study the combined effects of selenium on plants with other environmental parameters. We would like to assess the threshold of selenium beneficiary effects and toxicity. Additionally, the way of application of selenium to plants, which is the most safe for the nature will be assessed. And also, to determine which cultivated plants have ability to assimilate selenium to be the most suitable for the human nutrition.

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Antibacterial activity of *Mentha piperita* L. (peppermint) from leaf extracts – a medicinal plant

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ABSTRACT

In the present study, we evaluated the antibacterial activity in the leaf extracts of *Mentha piperita* L. against pathogenic bacteria like *Bacillus subtilis*, *Pseudomonas aureus*, *Pseudomonas aerogenosa*, *Serratia marcesens* and *Streptococcus aureus*. The aqueous as well as organic extracts of the leaves were found to possess strong antibacterial activity against a range of pathogenic bacteria as revealed by *in vitro* agar well diffusion method. The ethyl acetate leaf extract of *Mentha piperita* showed pronounced inhibition than chloroform, petroleum ether and water, leaf extracts being more on *Bacillus subtilis*, *Pseudomonas aerogenosa* than *Streptococcus aureus*, *Pseudomonas aureus* and *Serratia marcesens*.

Keywords: *Mentha piperita* L., antibacterial activity, leaf extracts.

IZVLEČEK

ANTIBAKTERIJSKA AKTIVNOST LISTNIH EKSTRAKTOV POPROVE METE (*Mentha piperita* L.)

Ocenjena je bila antibakterijska aktivnost listnega ekstrakta poprove mete (*Mentha piperita* L.) na patogene bakterije *Bacillus subtilis*, *Pseudomonas aureus*, *Pseudomonas aerogenosa*, *Serratia marcesens* in *Streptococcus aureus*. Tako vodni ekstrakti, kot ekstrakti dobljeni z organskimi topili so imeli močan protibakterijski učinek proti vrsti patogenih bakterij v agarju po *in vitro* metodi. Listni ekstrakt poprove mete, dobljen z etilnim acetatom je imel bolj izrazit učinek kot ekstrakti dobljeni s kloroformom, etrom ali vodo, bolje so učinkovali na *Bacillus subtilis* in *Pseudomonas aerogenosa* kot na *Streptococcus aureus*, *Pseudomonas aureus* in *Serratia marcesens*.

Ključne besede: *Mentha piperita* L., antibakterijska aktivnost, listni ekstrakti

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1 INTRODUCTION

Higher and aromatics plants have traditionally been used in folk medicine as well as to extend the shelf life of foods, showing inhibition against bacteria, fungi and yeasts (Hulin et al., 1998). *Mentha piperita* L. (peppermint) is a medicinally important plant that belongs to the family Labiate (Kirethekar and Basu, 1985). Peppermint is a non-native herbaceous plant, it is a perennial, which can reach 100 cm in height (40 inches) has four-sided stem. The leaves are stalked opposite and toothed. The flower are irregular in shape, they are pinkish or purplish (Clark and Menory, 1980). Peppermint leaves contains about 0.5-4 % volatile oil that is composed of 50-78 % free menthol, monoterpene, menthofurane and traces of jasmine (0.15 %) to improve the oils quality remarkably (Dew and Evans, 1984). Peppermint is largely cultivated in Indiana, Mexican and California for the production of peppermint oil. Peppermint oil or peppermint tea is often used to treat gas and indigestion; it may also increase the flow of bile from the gall bladder Mimica et al. (2003) and Forster (1996). Peppermint oils relaxing action also extended to tropical use, when applied tropically it acts as counterirritant and analgesic with the ability to reduce pain and improve blood flow to the affected area. Peppermint oil and menthol have moderate antibacterial effects against both gram-positive and gram negative bacteria Diaz et al. (1988). Peppermint extracts are bacteriostatic against *Streptococcus pyrogens*, *Streptococcus aureus*, *Streptococcus pyrogens*, *Serratia marcescens*, *E.coli* and *Mycobacterium avium* (Gotshall, 1949). Peppermint is also found to have antiviral and fungicidal activity (Chaumont and Senet, 1978). Menthol is virucidal against influenza, herpes and other viruses. Aqueous extracts of peppermint leaves were antiviral against influenza A, newcastle disease virus in egg and cell culture system Hirobe (1994) and Alkofahi et al. (1990). Menthol and peppermint oil are fungicidal against *Candida albicans*, *Aspergillus albus* and *Dermatophytic* fungi. However, to the best of our knowledge, no serious efforts have been made to test the antibacterial properties of *Mentha piperita* so far. In the present study we established antimicrobial activity of *Mentha piperita* against pathogenic bacteria. The study confirms that both aqueous as well as organic solvent leaf extracts possess strong antibacterial properties against various pathogens viz., *Bacillus substillus*, *Pseudomonas aureus*, *Pseudomonas aerogenosa*, *Serratia marcesens* and *Streptococcus aureus*.

2 MATERIALS AND METHODS

2.1 Preparation of leaf extracts

Apparently healthy plants were collected, washed thoroughly in tap water and dried at dark room temperature for 15 days. The leaves was powdered and extracted following the published procedure with slight modification as standardized in our lab Alade and Irobi, (1993); Essawi and Srour (2000). The powdered material was soaked in petroleum ether, chloroform, ethyl acetate, ethanol, methanol and water by keeping it in a shaker for 3 days. The extracts were filtered through cheesecloth and the extracts were reduced to 10 % of its original volume. The filtrate organic solvents were concentrated in vacuum using a rotary evaporator, while aqueous extracts were dried using water bath.

2.2 Separation of the compounds

The menthol present in *Mentha piperita* leaf extracts were analysed for qualitative by using thin layer chromatography which is commercially available TLC, aluminum sheets with silica

gel 60F₂₅₄ were used. The isolation separation of menthol was done by using the procedure of Stahl (1964).

2.3 Inoculums

The test microorganisms *Bacillus subtilis*, *Pseudomonas aureus*, *Pseudomonas aerogenosa*, *Serratia marcesens* and *Streptococcus aureus* were obtained from culture repository of Best Biotech culture collection, Bangalore, India. The organisms were inoculated onto NB (Nutrient Broth), (0.5 % Peptone, 0.5 % Sodium Chloride, 0.15 % Yeast extract; pH 7.4) and incubated at 37 °C for overnight. The bacterial cells were harvested by centrifuging at 5000g for 15 min. The pellet formed was washed twice with PBS (Phosphate Buffer Saline), (10 mM Sodium Chloride, pH 7.4) and the cells were counted by haemocytometer. The bacterial cells were diluted to approximately 10⁵ CFU/ml before use (Owais et al., 2005).

2.4 Determination of antibacterial activity:

The antibacterial activity of the leaf extracts was determined using agar well diffusion method following published procedure with slight modification (Perez et al. 1990). Nutrient agar was inoculated with the given microorganisms by spreading the bacterial inoculums on the media. Wells (8 mm diameter) were punched in the agar and filled with plant extracts. Control wells containing neat solvents (negative control) or standard antibiotic solution (positive control) viz., Chloromphenicol (100 µg/ml) were also run parallel in the same plate (Valsaraj et al., 1997). The plates were incubated at 37 °C for 18 h. The antibacterial activity was assessed by measuring the diameter of the zone of inhibition for the respective drug. The relative antibacterial potency of the given preparation was calculated by comparing its zone of inhibition with that of the standard drugs viz., Choloramphenicol.

2.5 Statistical analysis

The resultant clear zones around the discs were measured in mm. The antibacterial activity of plant extracts were indicated by clear zones of growth inhibition. Five replicates were maintained for each treatment. The data were subjected to statistical analysis as per the method of Gomez and Gomez (Gomez and Gomez, 1976).

3 RESULTS

We used both polar as well as non-polar solvents for the extraction of active components from the leaves of *Mentha piperita* plant. The antibacterial activity of the *Mentha piperita* was assessed using the agar well diffusion method by measuring the diameter of growth inhibition zones and its subsequent concentration was tabulated (Table. 1). The results shown in (Table 1 and Figure 1) indicate that the aqueous and polar solvent extracts possessed strong antibacterial activity. In ethyl acetate, chloroform and water the highest antibacterial activity was retained in 50 µl and 100 µl concentration of leaf extracts. We found that both aqueous as well as ethyl acetate extracts of leaves were successful in killing the bacteria in a dose dependent manner. The MIC (Minimal inhibitory Concentration) for the aqueous extracts was found out to be 10 mg/ml for *Bacillus subtilis* and *Pseudomonas aerogenosa*. While *Serratia marcesens* and *Streptococcus aureus* required about 0.25 mg/ml of the crude extract for effective killing. On the other hand *Pseudomonas aureus* was inhibited at the dose of 0.35 mg/ml of the crude extract. The zone of inhibition assay results demonstrated that the ~8 mg of crude leaf extract was able to produce same effect as that of 20 µg of Choloromphenicol (data not shown). Beside the 50 µl concentration of leaf extracts, the 100 µl concentration of leaf extracts was found to possess maximum inhibition, although, overall potency was about half to that of the 50 µl concentration

of leaf extracts. (Table 1 and Figure 1,2). Upon chemical analysis, the extracts were found to possess glycosides and alkaloids. The extracts were found to be rich in menthol compound as revealed by specific assay. The presence of menthol was further confirmed by thin layer chromatogram, which showed presence of green fluorescent spots in the extracts of chloroform (Figure 3). The ethyl acetate extracts had very large zones of inhibition ranging from 9 mm and it also showed high degree of inhibition against *Bacillus subtilis*, *Pseudomonas aerogenosa*, *Streptococcus aureus* than *Pseudomonas aureus* and *Serratia marcesens*. The chloroform leaf extracts showed moderate inhibition against *Bacillus subtilis*, *Pseudomonas aureus* and *Streptococcus aureus* than *Pseudomonas aerogenosa* and *Serratia marcesens* But the petroleum ether leaf extracts exhibited less inhibition against *Bacillus subtilis*, *Pseudomonas aureus* and *Serratia marcesens* than *Streptococcus aureus* and *Pseudomonas aerogenosa*.

4 DISCUSSION

We used both the aqueous and polar solvents for the extraction of active components from leaves of the plant. The result of the study reveals that an aqueous and polar solvent was actively against the strains of the bacteria that are common cause of infections. *Mentha piperita* shows significant activity as because the leaf contains many potent compounds such as menthol, menthone, menthyl acetate, menthofuran, and limnone (Fleming, 1998). These compounds have higher medicinal value especially in the treatment of dyspepsia, epigastric bloating, impaired digestion, eructations, and flatulence, tropically used to relieve nasal congestion in the common cold and itch relieving used as tropic protective agents Alkofahi et al. (1990). The biologically active compounds are screened by dissolving the crude powder on various solvents respective to the solubility of the compound specific solvents confirmed by the TLC (data not shown). The antibacterial activity was expressed at varying degrees with the activity being both strain and dose dependent. The various crude extracts of *Mentha piperita* showed significant activity against all the bacteria tested. Similar to our result, the biological activity of *Mentha piperita* against the pathogenic bacteria were reported by (Deans and Baratta, 1998). Based on that, we used the three different solvent extracts of leaves of *Mentha piperita* showed activity against all bacteria at all dosages. The leaf extracts of *Mentha piperita* exhibited antibacterial activity only in ethyl acetate, petroleum ether, chloroform, methanol and aqueous extracts against the bacteria tested in agar well diffusion method at 50 μ l -100 μ l concentration by the following method of Valsaraj et al. (1997). We observed maximum activity at 100 μ l concentration against *Pseudomonas aerogenosa*, *Streptococcus aureus*, *Bacillus subtilis* than *Serratia marcesens* and *Pseudomonas aureus*. The present reports indicates that increased lipophilic compounds are extracted using the petroleum ether, chloroform and methanol increased the suspended higher compounds in the above solvents as stated by Tomas et al. (1988). We subjected the leaf extracts of different solvents to thin layer chromatography and column chromatography to identify the compounds dissolved in the solvents and quantifies the compounds using spectrophotometer techniques followed by the previous work (Essawi and Srour, 2000). The present work was similar to (Deans and Baratta, 1998) shows that the compounds from *Mentha piperita* possess potent antimicrobial activity and suggesting that the *Mentha piperita* leaf extracts should

contains the effective active constituents responsible for eliminating the bacterial pathogens. Finally, it can be concluded that the active chemical compounds present in *Mentha piperita* should certainly find place in treatment of various bacterial infections. The results from the present study are very encouraging and indicate this herb should be studied more extensively to explore its potential in the treatment of infectious diseases as well.

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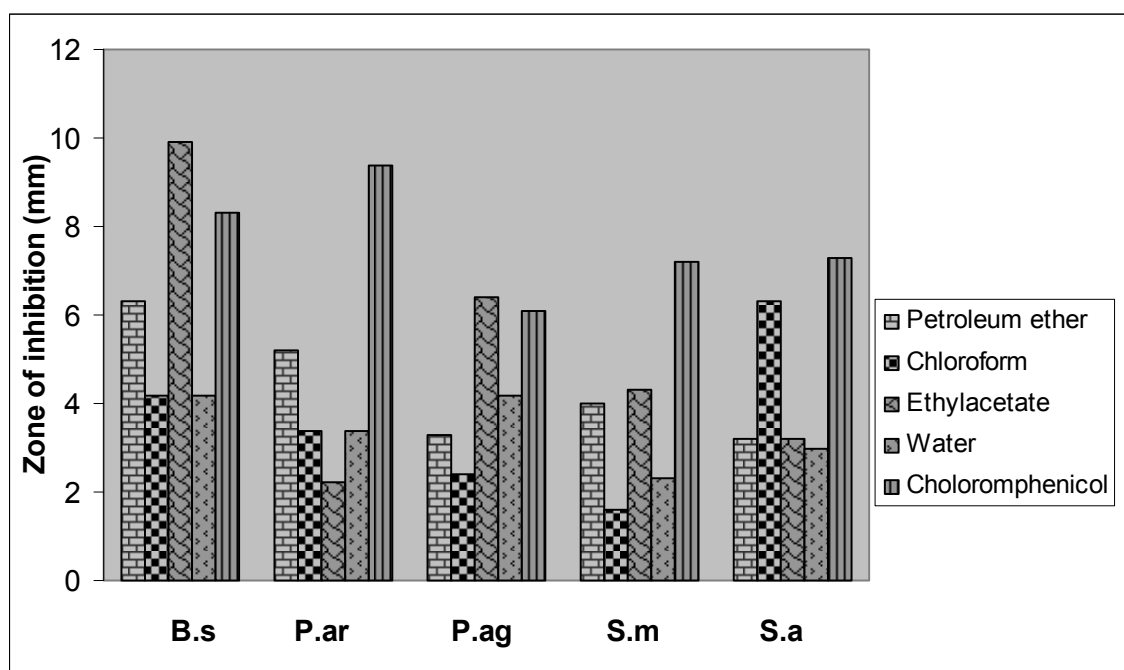
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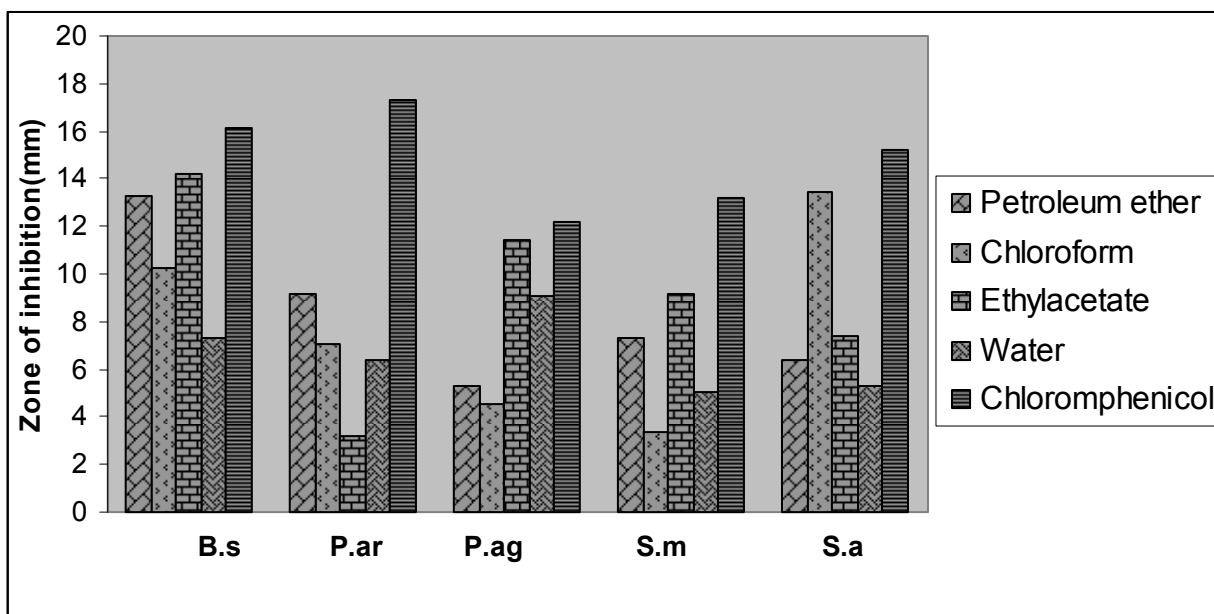
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The antibacterial activity of leaf extracts of *menthapiperita* was screened at 50 μ l concentration against B.s = *Bacillus subtilis*, P.ar = *Pseudomonas aureus*, P.ag = *Pseudomonas aerogenosa*, S.m = *Serratia marcesens* and S.a = *Streptococcus aureus*. The leaf extracts were dissolved in different solvents a. Petroleum ether b. Chloroform c. Ethyl acetate d. Water e. Control – Chloromphenicol.

Figure 1: Antibacterial activity of leaf extracts of *Mentha piperita* was screened at 50 μ l concentration



The antibacterial activity of leaf extracts of menthapiperita was screened at 100 µl concentration against B.s = *Bacillus substillus*, P.ar = *Pseudomonas aureus* P.ag = *Pseudomonas erogenosa*, S.m = *Serratia marcesens* and S.a = *Streptococcus aureus*. The leaf extracts were dissolved in different solvents Viz a. Petroleum ether b. Chloroform c. Ethyl acetate d. Water e. Control – Chloromphenicol

Figure 2: Antibacterial activity of leaf extracts of *Mentha piperita* was screened at 100 µl concentration

Table 1: Antibacterial activity of the active components present in the leaf extract of *Mentha piperita* against various microorganisms

Extract	Zone of inhibition in (mm) (Mean ± SD)									
	50µl					100 µl				
	<i>B.subtilis</i>	<i>P.aureus</i>	<i>P.aerogenosa</i>	<i>S.marscens</i>	<i>S.aureus</i>	<i>B.subtilis</i>	<i>P.aureus</i>	<i>P.aerogenosa</i>	<i>S.marscens</i>	<i>S.aureus</i>
Pet. Ether	6.3±0.17	5.2±0.21	3.3±0.13	4.0±0.41	3.2±0.31	13.2±0.39	9.2±0.16	5.3±0.33	7.3±0.33	6.45±0.32
Chloroform	4.2±0.25	3.4±0.23	2.4±0.28	1.6±0.28	6.3±0.32	10.2±0.21	7.1±0.24	4.5±0.32	3.4±0.30	13.4±0.31
Ethyl acetate	9.9±0.28	2.3±0.27	6.4±0.30	4.3±0.41	3.2±0.25	14.2±0.17	3.2±0.20	11.4±0.29	9.2±0.26	7.4±0.30
Water	4.2±0.25	3.4±0.23	4.2±0.23	2.3±0.35	3.0±0.33	7.3±0.27	6.4±0.28	9.1±0.56	5.08±0.23	5.26±0.28
Chloromphenicol	8.3±0.23	9.4±0.23	6.1±0.14	7.2±0.29	7.3±0.30	16.1±0.18	17.3±0.37	12.2±0.33	13.2±0.37	15.2±0.23

The antibacterial activity of leaf extracts of *Mentha piperita* against various bacterial strain was developed by agar diffusion method. The active component of the leaves of *mentha piperita* were extracted with different solvents. The solid residues in respective solvents at 10mg/ml concentration. The well bored in bacterial culture plates were filled with 100 µl of suspension. (~2mg crude extract). The negative control wells were exposed with the neat solvent. Each value represents mean of five different observations ± S.D.

Agrovoc descriptors: organic agriculture; alternative agriculture; traditional uses; surveys; farm surveys; sustainability; human behaviour; value systems; motivation; environmental protection; technological changes

Agris category code: F08, E20, E51

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The characteristics of conventional and organic farmers in Podravska region¹

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ABSTRACT

The paper presents the results of a questionnaire survey on adoption of organic farming in Podravska region. The survey was conducted on the sample of 58 organic farms and 99 conventional farms in the year 2005. The results show certain differences among both groups of farms in information adoption and attitudes towards nature sustainability. Both groups of farms use different information sources, and are members in different associations. Also the level of environmental awareness is higher by organic farmers but in many other characteristics the differences are not significant. The analysis of future plans of conventional farmers also revealed a significant proportion of farms which are undecided regarding the conversion of their farms to organic agriculture in the next 10 years.

Key words: attitudes, ecological agriculture, agri-environmental schemes

IZVLEČEK

ZNAČILNOSTI KONVENCIONALNIH IN EKOLOŠKIH KMETOV V PODRAVJU

Prispevek podaja rezultate anketne raziskave o sprejemanju ekološkega kmetijstva v Podravju. Raziskava je zajela vzorec 58 ekoloških in 99 konvencionalnih kmetij v letu 2005. Rezultati kažejo določene razlike med obema preučevanima skupinama kmetij glede iskanja in uporabe informacij, in odnosa do trajnosti naravnega okolja. Tako kmetje iz posamezne skupine uporabljajo različne informacijske vire in so člani različnih združenj. Tudi raven okoljske zavesti je višja pri ekoloških kmetih, pri mnogih ostalih značilnostih pa ni bilo opaziti signifikantnih razlik. Analiza prihodnjih načrtov konvencionalnih kmetov je razkrila, da je velik delež takih, ki so neopredeljeni glede možnosti konverzije svoje kmetije v ekološko v prihodnjih 10 letih.

Ključne besede: vedenje, ekološko kmetijstvo, kmetijsko okoljski ukrepi

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1 INTRODUCTION

Organic farming presents a possibility for part of Slovene's farms to continue with their activity and to gain a satisfactory farm income when combined together with payments from agricultural policy measures. Slovenia plans to still increase the number of organic farms and their production areas (Strategija razvoja..., 2006). The series of surveys in 2003 and 2004 (Jelen, 2003; Kejžar, 2003; Serjun, 2005; Stropnik, 2003) also found out that demand for Slovene organic products is still exceeding the existing supply of all kind of organic products in all regions. The above summarises the reasons why it is important to study the adoption and the key factors influencing the decision about starting organic farming.

In Slovenia the organic farming started to receive agricultural policy support already in the year 1999. In 2001 the Slovene agri-environment programme (SAEP) was implemented and organic farmers were legitimate to direct payments for the measures from this programme. The first payments within the SEAP were paid out in 2002. Following this, the numbers of organic producers has increased every year. For illustration, 41 organic producers applied for subsidies in 1999, as in 2004 there were already 1,489, what represents 2% of all Slovenian farms (Škerbot, Uranjek, 2005).

Our main research objectives were to analyse the agricultural situation on selected agricultural holdings (conventional and organic) in the selected region (the north-eastern part of Slovenia, the Podravska region) and to compare the results between organic farmers and conventional farmers. More specifically, our study tried to find answers to the following research questions:

- Are there significant differences between organic farmers and conventional farmers regarding the agricultural situation on holdings (farm size, education level, income level, number of family members, memberships of professional associations etc.)?
- Are the relations and views to nature, concerns about future generations, environment...significant different between organic farmers and conventional farmers?

The analyses of factors that influence farmers' adoption of innovations, as well as analyses of the differences between organic and conventional farming have started in late 90ies when the participation of farmers in the EU member states in agri-environmental programmes became more widespread. In one of the earlier analyses Wilson (1997) grouped factors influencing the adoption of certain agri-environmental scheme in "scheme factors" (e.g. payments) and "farmer factors" (e.g. age of farmer). In his analysis he proved that the strongest factor influencing the decision for adopting a certain scheme/s is farm size, where the medium size farms were most interested to adopt. That the farm size is one of the most important factors also indicated the analysis of Diederer et al (2003), Chaves (2001). On the other hand Burton in his analysis (2003) found farm size as insignificant factor. Other factors which also showed some influence on adoption of either organic farming or certain agri-environmental schemes are: education, farmer's attitude towards environment, sources of information and income situation of the farm (Wilson, 1997, Diederer, 2002, Burton 1999 and 2003, McCann and Sullivan, 1997).

2 METHODS

The study sample was selected from farms in the northern part of Podravska region, where both organic and conventional farms exist side by side. The reason for selecting particular local municipalities for farm sampling was determined by the fact that the agricultural advisory service for both types of farms is offered by the same institution – the Maribor Agricultural Institute.

The sampling of organic farms was done on the basis of the membership list of the Association of Organic Farmers of Podravje, which provided us with the list of 78 farms (including farms in conversion). From this list all farms which exceeded the threshold of subsistence farms (1 ESU⁴) were selected. So we got a list of 72 farms, from which 6 had already stopped to farm. Finally 59 agreed to be interviewed.

For conventional farms, a stratified sample by ESU size classes was provided from the National Statistical Office (SURs). In each class (see table 2 for class sizes) 40 conventional farms were selected to enable a final sample size of 33 farms per class. The initial survey was performed during the winter 2005 for both groups.

Because of the time difference between database establishment (year 2000 for the purpose of national agricultural census) and realisation of our survey, the selected sample didn't prove to be adequate. There were some farms in the sample that had stopped with farming, or its economic size changed. So additional sampling for conventional farms was necessary and a second round of interviews was done in late summer 2005. Thus, for inferences to the regional farm population, the sample needed to be weighted.

Table 1: Number of farms and their distribution in the region and in the planned sample (Source: SURs 2006; regional data per 31. 12. 2004)

		Region	Sample
Conventional farms	Subsistence (<1 ESU)	801	0
	Small (1<2 ESU)	680	33
	Medium (2<6 ESU)	778	33
	Large (6 ESU and more)	560	33
Sub-total		2819	99
Organic farms*	Subsistence (<1 ESU)	6	0
	1 ESU and more	66	66
Sub-total		72	66

* Including farms in conversion; 1 ESU = total standard gross margin on a farm divided by 1200 Euros

Table 2: Distribution of sample farms by economic size classes in realised survey

	Size classes				Total
	Subsistence	Small	Medium	Large	
Conventional farmers	15	18	33	32	99
Organic farmers	2	5	29	22	58
Total	17	23	67	49	157

The personal interviews were based on fully structured questionnaires that were pre-tested in this, and five further regions in other EU accession countries. The questionnaire consisted of 250 variables grouped in seven sections:

⁴ ESU - European Size Unit is a measure of the economic size of a farm business based on the gross margin imputed from standard coefficients for each commodity on the farm. The application of these standard coefficients results in the Standard Gross Margin (SGM) for a farm or group of farms (1 ESU = 1200 EUR).

- role of formal institutions and information sources,
- general attitudes towards environment,
- conversion to organic agriculture,
- characteristics of the farm,
- characteristics of farm manager and his family,
- finance and market development, and
- specific attitudes - behavioural relationships.

The opinions variables of interviewees were measured on semantic differential scales ranging from 1 to 7.

In this article we present the results of the analyse of differences between organic and conventional farms in farm size, education, information sources about agricultural production methods, dependency on farm income and personal attitudes towards environmental and economic factors.

The quantitative analysis of the survey data was done by means of descriptive statistical analysis, and research questions were tested by use of chi-square test for two independent variables.

3 EMPIRICAL RESULTS

The farm population

The general characteristics of total farm population as well as organic farms in the survey area compared to the whole country are presented in the tables 3 and 4. The regional data on organic farms were provided by the organic farmers association of Podravje.

Within the survey we defined as organic farmers those farmers, who already received certificate of organic agriculture, are in the process of conversion, and as conventional farmers are indicated those farmers who are farming in conventional way.

Table 3: Total and organic farm populations in Slovenia. 2000 and survey region (SURS, 2002)

	Country	Survey area
Total no. farms*	86,427	3,078
No. organic farms**	620	78
% of total no. farms	1 %	3 %
Total hectares farmed	537,249	15,539
Hectares farmed organically	5,425	903
% of total hectares farmed	1 %	5.8 %
Average size of agricultural holdings* (ha)	6.2	5.0
Average size of organic holdings** (ha)	8.8	11.6
Farm types specialist crops	13,177	463
Farm types specialist livestock	24,312	650
Mixed farm types	48,942	1,865
Individual farms	86,336	3,075
Corporate farms	131	3

* Excluding household plots. ** Including farms in conversion.

Table 4: Size structure of farms in survey area, 2000 (SURs, 2002)

Number of predominately subsistence farms*	964
Number of small, but trading farms (<= 5 ha)	1119
Number of medium sized farms	595
Number of large farms (>= 10 ha)	400
Total	3078
Total excluding predominately subsistence farms	2114

* E.g. if a farm sells only a minor part of its products to neighbours, this still counts as being predominately subsistent.

Farms in Slovenia are mostly of mixed type, what is a consequence of natural conditions and big share of absolute grassland. Also in our sample we can find the same situation with 53% of organic farmers' and 61% of conventional farmers' farms being of mixed crop-livestock type. The next most frequent farm type among conventional farmers are specialised field crops farms with 13% and among organic farmers specialised grazing livestock farms (38%). All the other types are presented with small share of farms.

Full time family farms present 74% of all organic farmers' farms in a sample and almost 52% of conventional farmers' farms. Organic farmers are thus more professional engaged in agriculture.

Table 5: Descriptive statistics for the sample

Variable	Organic farmers (58)		Conventional farmers (99)	
	Mean	SD	Mean	SD
Age of farm manager	48.5	11.274	54.4	12.181
Income earned from farming (%)	32.86	19.94	39.43	25.686
Farm size (ha)	3.71	2.209	4.53	1.72
Predominant farm type	Mixed crop – livestock (53%)		Mixed crop – livestock (61%)	

SD = Standard Deviation;

Among questioned farm managers most of them were males. Women presented 33% of questioned organic farmers and 27% of conventional farmers. 69% of organic farmers and approximately 48% of conventional farmers are full-time engaged in farming. The rest are part-time farmers. Regarding the age structure of farmers/managers there are noticeable differences in age structure of farmers between organic farmers and conventional farmers. By the organic farmers the younger age groups show higher share of managers as by conventional farmers and opposite. In last two classes (older than 56 years) we can find almost half (48.5%) of conventional farmers and only 25.9% of organic farmers, what indicates that younger farmers are more interested in adopting organic farming, what is also confirmed by the chi-square test ($p=0.02$). More than 86% of organic farmers and 94% of conventional farmers have children, so they can expect that their family farming will continue.

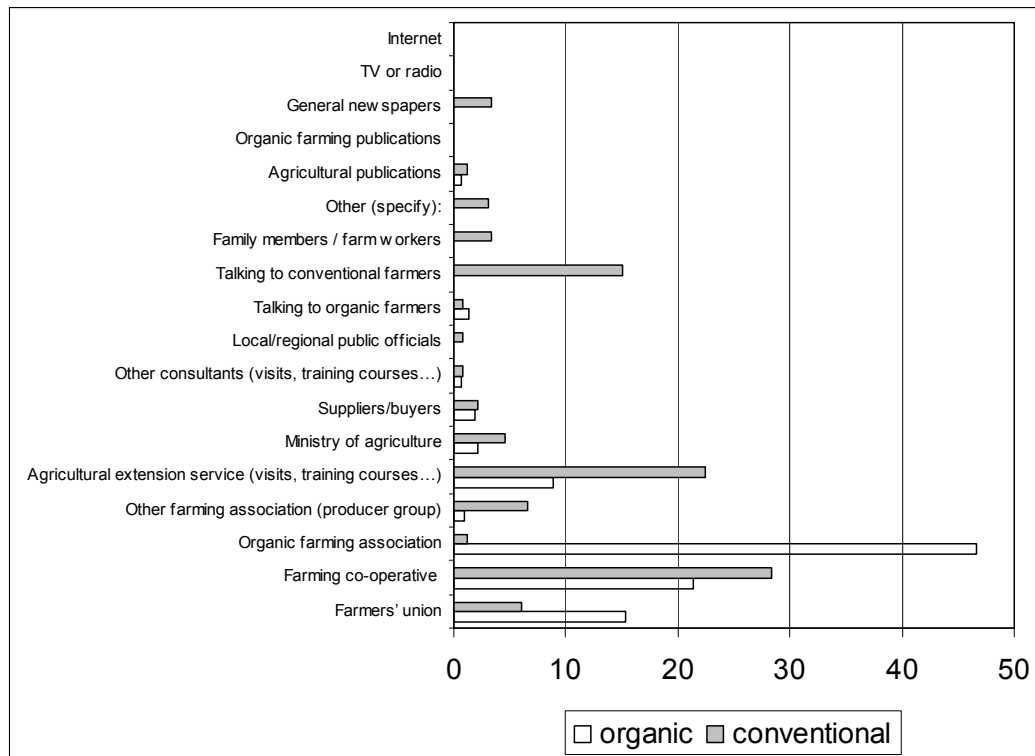
For Slovenian farmers is typical that they gained their farming experiences from on-farm training and different courses organized mostly by Agricultural Advisory Service. The questioned farmers also show this pattern with the highest share of farmers in both samples having only on-farm training with the combination of their own learning (from books and practical experiences) or just on-farm training. Frequent combination is also on-farm training, own learning and some courses. Only 20.7 % of organic farmers and 17.2 % of conventional farmers have an agricultural high school of higher level of agricultural training. The general education of questioned farmers can be, in comparison to results from Agricultural survey data from 2000 (SURS, 2002), valued as good. Organic farmers are having higher level of education as conventional farmers, but the difference is not significant ($p=0.1$), so it can't be presumed that the level of education influences the decision for conversion. Most of questioned farmers of both groups have secondary education. Bigger difference is regarding farmers with finished college or university. Around 17% of organic farmers and 8% of conventional farmers finished college or university.

3.2 Role of formal institutions and information sources

Farmers are generally not very often members of different associations and organisations, and when they are members, then this organisations are often of professional nature. In the analysed case the organic farmers are in high share members of organic farming association (86.2 % of them), while conventional farmers are mostly members of farming co-operative but with much smaller share (29.3 % of them). Results also show that organic farmers are more often attending meetings of their organisations as conventional farmers. The highest share of organic farmers attends meetings of Organic farming association and farming co-operative meetings. Conventional farmers attend fewer meetings, mostly of farming co-operatives or other formal or informal farmer groups (mainly Cattle breeders associations and Agricultural and Forestry Chamber).

Farmers get general information on agriculture from different sources. Organic farmers get them mostly from organic farming associations, while the most important source for conventional farmers are farming co-operative and agricultural extension service. For conventional farmers important source of information is also talking with other conventional farmers and Farmers' union.

Most important general information source for organic farmers are Organic farming associations and agricultural advisory service. All the other sources are not so often used. Conventional farmers get general information on agriculture mostly from agricultural advisory service and from other conventional farmers and farming co-operatives. The fourth most important information source for conventional farmers are agricultural publications. Modern communication sources are almost not in use in both groups.



Picture 1: Number of farms which use specified information sources

Both groups of farmers answered also about their main source of information on organic farming. As expected the most important source of information on organic farming for organic farmers are Organic farming associations, less frequent sources are also farmers' union, farming co-operatives and agricultural advisory service. Conventional farmers get information about organic farming mostly from other conventional farmers, from agricultural advisory service and farming co-operatives. It is interesting that both groups of farmers don't use modern information sources as internet and TV or radio. Media source were not very often mentioned. Newspapers and agricultural publications are used more often from conventional farmers as from organic farmers.

Regarding the opinion about organic farming, conventional farmers in general think that majority of farmers have more or less good opinion about it. The organic farmers opinion is less optimistic and there is a significant difference among both groups ($p=0.001$).

3.3 Successor situation

Assured successor is one of the important factors for long term family farming sustainability. Successor is already known on 39.7% of organic farmer's farms and on 40.4% of conventional farmer's farms and successor status has not significance for adopting the organic farming. More than 20% of organic farmer's and conventional farmer's farms don't have assured successor and on the rest of farms it is not applicable because of different reasons: children are still too young, farm is owned by parents, farm is small, farmer's wife is against potential successor, farmer is not owner of the farm and farm is already on sale.

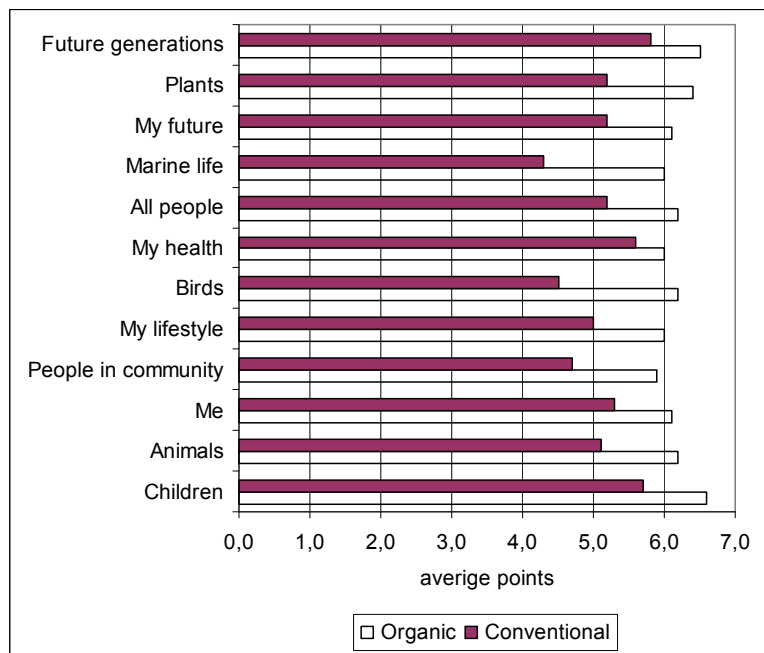
On the question how successor situation influences on the decision whether to convert to organic farming or not the answers among organic farmers group were bit surprising, because big share of them answered that successor situation is not important at all for their decision. So the decision to convert is not much connected with future but with present situation and with present generation. Only smaller share of organic farmers put bigger importance of the successor situation in decision process. Conventional farmers predominantly consider successor situation as quite, slightly or even very important, but smaller part of them also as not important factor. The answers between the groups are significantly different ($p=0.001$).

3.4 Farmers attitude towards environmental and economic factors

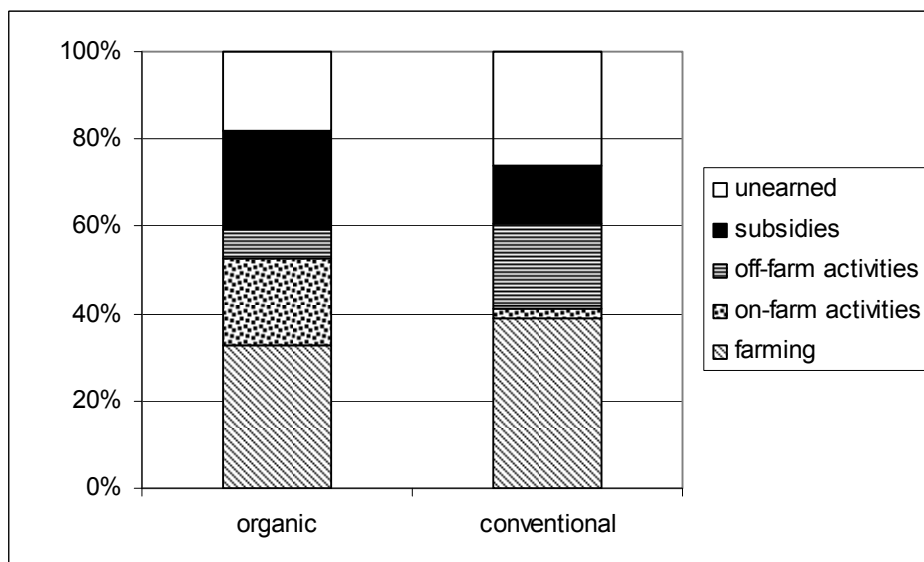
In general both groups are showing positive concern about their environment, but some differences in attitudes between organic farmers and conventional farmers are noticeable. Organic farmers concern significantly more ($p=0.001$) about most mentioned environmental problems as shows in the picture 2. Only in case of personal health and own personality (Me) the differences are not significant. The highest concern is in both groups given for their children and future generations. Organic farmers showed the lowest concern for other people in community, while conventional farmers have the smallest concern for marine life. The same situation is noticeable in their relationship with the natural environment. Both groups of farmers feel closely connected with a nature. The reason for this can be explained as the agricultural activities are very connected with nature and also depend on nature and natural conditions. Also here organic farmers are more narrowly connected with nature as a conventional farmers (average score 2.84 : 2.54).

Organic farmers and conventional farmers have in most cases from 25% to 50% of their farm's annual gross income from farming. In average conventional farmers have 39% of income from farming and organic farmers 32.9% (see table 5). The share of income from farming is not significant for decision to adopt organic farming.

Picture 3 present average distribution of different kind of incomes on interviewed farms. Comparing average amounts we can see that income from farming is more important for conventional farmers (but not significant), on farm activities bring significantly ($p=0.001$) more income on organic farmer's farms, off-farm activities contribute also to farm's annual gross income (more important for conventional farmers, but not significant), while subsidies present bigger part of farm income for organic farmers ($p=0.01$) as for conventional farmers. The reason can be found in fact that organic farmers get money also to implement other measures of Slovene Agri-Environment Programme (SAEP). Unearned income (i.e. social transfers) in average is also important for overall farm income (18.2% of total farm income for organic farmers and 26% for conventional farmers).

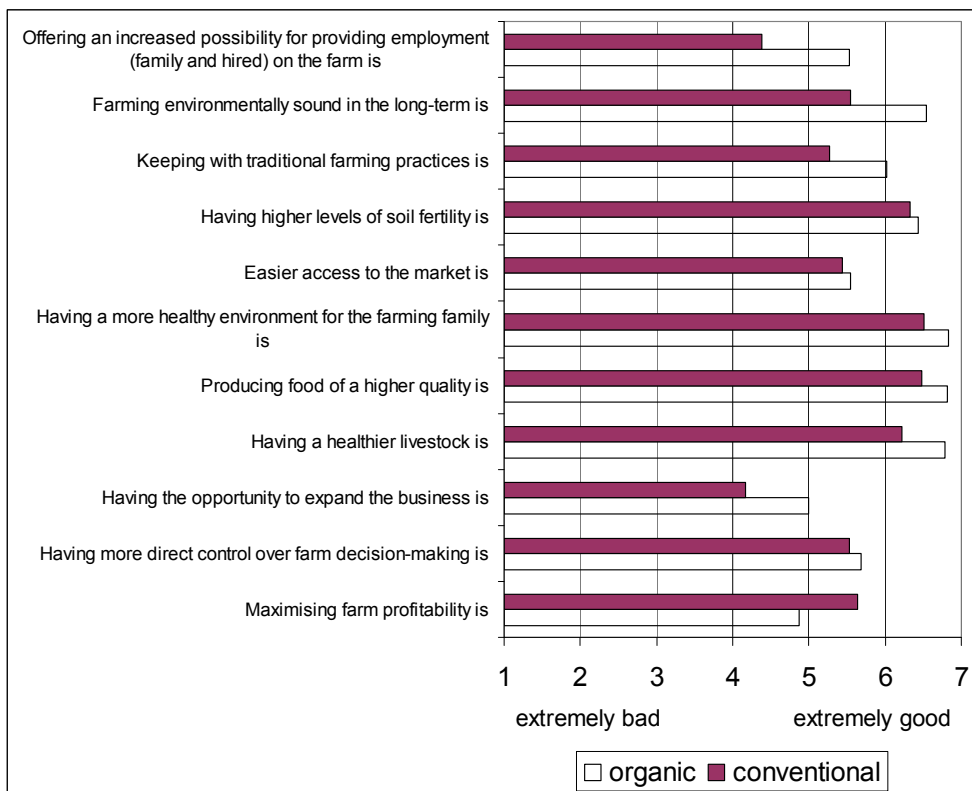


Picture 2: Average scores for different concerns regarding the environment



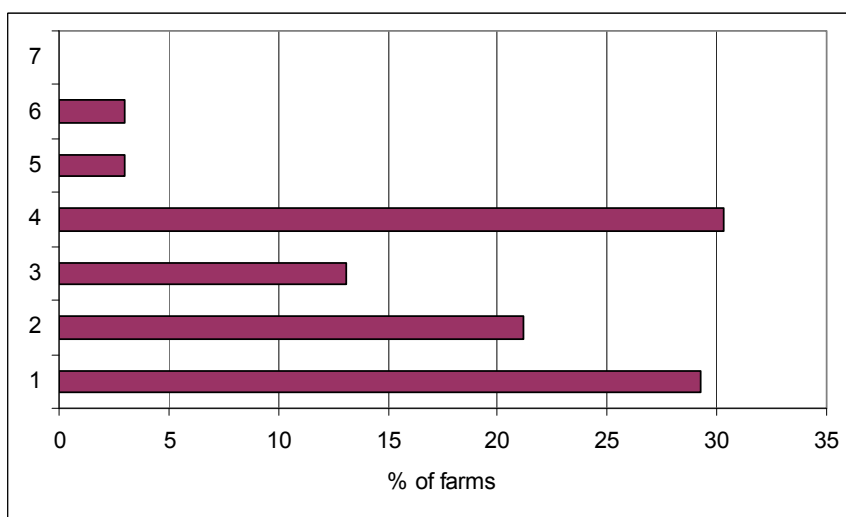
Picture 3: Average income structure on organic farmers' and conventional farmers' farms

Analysis of farmers' attitude towards environmental and economic factors of their business operations showed certain difference between organic farmers and conventional farmers. The organic farmers are assessing the listed environmental and economic factors higher than conventional farmers with the exception of profit maximisation. The statistically significant difference could be calculated by possibility for providing on farm employment and opportunity to expand the business.



Picture 4: Scores for personal importance of certain factors for farm managers

The results of the analysis also show that conventional farmers mostly do not plane to convert within the next 10 years or are not really certain about that option (score 4) (Picture 5). But on the other hand 50% of them stated that they will monitor the development within the organic production and they leave open the possibility to reconsider it.



Picture 5: Likelihood of conventional farmers to convert in next 10 years (1- strongly disagree, 7 – strongly agree)

Also the results on a question “What is the likelihood of you having a *farm appraisal* for investigating ‘organic farming’ as a possible alternative to conventional farming over the next 10 years?” show that 40% of conventional farmers consider it likely to

extremely likely. For all the other this is unlikely to extremely unlikely. This shows that there is a certain insecurity about the future development of conventional agriculture for small Slovene farms. And as official agricultural policy is for the future strongly promoting the stronger shift towards more sustainable and organic agricultural production the farmers do consider this option also in their future development alternatives.

4 CONCLUSIONS

Comparing the results to previously published analysis (Wilson, 1997, Diederon, 2002, Burton 1999 and 2003, McCann and Sullivan, 1997) we can see that our results in general confirm our hypothesis and previous findings that there are factors which distinguish organic from conventional farms as farm size, income structure, education, membership of professional associations, dependency on farm for income and personal attitudes towards environmental concerns, but some of them did not proved to be statistically significant (education, share of income from agriculture).

On the other side the successor factor proved to be more important to conventional farmers as to organic farmer what contradicts to what was stated by research of Wilson (1997), Potter and Lobley (cit. by Wilson, 1997). This result can be partly explained with the fact that for one half of questioned farmers the successor question is not an issue, as the farmers are still young or they already have the successor. Though there is no significant difference in the successor situation structure the difference in importance of successor factor my rise from the fact that smaller conventional farmer generally feel higher level of insecurity regarding their farm future, mostly because the next generation has better income opportunities in off farm activities.

Though, investigating the motivational and financial structures of organic farmers lets us conclude that adopters could partly not realise their expectations or they decided for organic production as this enabled them to get some extra income from subsidies not forcing them to significantly change their production techniques. They have mostly been producers of milk and cattle, which can often not earn an extra margin on the organic market, pointing towards a mismatch of supply and demand not only in quantity, but also in the kinds of products as the demand for organic products still exceeds what is offered in many product categories (Akcijski načrt..., 2005).

Considering this and the results of the presented survey we can anticipate, that the future adoption of the organic agriculture is dependant on one side on the future development of the demand for organic food products and its processing possibilities (most important segment are animal products), and on the other side the future development of economics of conventional and organic agriculture especially for small size part time farms which are typical for Slovenia and are at the moment still continuing with its low intensity conventional production.

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Agrovoc descriptors: aesculus; lepidoptera; geographical distribution; identification; monitoring; pest insects; infection

Agris category code: H10, L20

COBISS code 1.01

The Occurrence of some Lepidopterous species on the horse chestnut (*Aesculus hippocastanum* L.) at Istanbul-Belgrad Forest in Turkey

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ABSTRACT

The occurrence of some Lepidopterous species was determined between 2004 and 2005 on the horse chestnuts of Istanbul-Belgrad Forest in Turkey. The following eleven pests of the horse chestnut were recorded: *Cameraria ohridella* (Deschka & Dimic, 1986) (Gracillariidae), *Archips crataegana* (Hübner, 1799), *A. podana* (Scopoli, 1763), *A. xylosteana* (Linnaeus, 1758) (Tortricidae), *Crocallis elinguaris* (Linnaeus, 1758), *Ennomos quercaria* ([Hübner], [1812]), *E. quercinaria* (Hufnagel, 1767), *Erannis defoliaria* (Clerck, 1759), *Operophtera brumata* (Linnaeus, 1758), *Pachycnemia hippocastanaria* (Hübner, 1799) (Geometridae) and *Amphipyra pyramidea* (Linnaeus, 1758) (Noctuidae).

Key words: Lepidoptera, horse chestnut, pests, Istanbul, Turkey.

IZVLEČEK

POJAV NEKATERIH VRST LEPIDOPTER NA DIVJEM KOSTANJU (*Aesculus hippocastanum* L.) V GOZDU ISTANBUL-BELGRAD V TURČIJI

V letih 2004 in 2005 je bil ugotovljen pojav vrst Lepidopter na divjem kostanju v gozdu Istanbul-Belgrad v Turčiji. Ugotovljenih je bilo naslednjih enajst škodljivcev: *Cameraria ohridella* (Deschka & Dimic, 1986) (Gracillariidae), *Archips crataegana* (Hübner, 1799), *A. podana* (Scopoli, 1763), *A. xylosteana* (Linnaeus, 1758) (Tortricidae), *Crocallis elinguaris* (Linnaeus, 1758), *Ennomos quercaria* ([Hübner], [1812]), *E. quercinaria* (Hufnagel, 1767), *Erannis defoliaria* (Clerck, 1759), *Operophtera brumata* (Linnaeus, 1758), *Pachycnemia hippocastanaria* (Hübner, 1799) (Geometridae) and *Amphipyra pyramidea* (Linnaeus, 1758) (Noctuidae).

Ključne besede: Lepidoptera, divji kostanj, škodljivci, Carigrad, Turčija.

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INTRODUCTION

The general area of Turkey is 77.056.192 ha. The forested area is 21.188.747 ha, so 27.5% of the land in the country is covered with forests (Anonymous, 2006a). The Belgrad Forest corresponding to 0.03% of forested areas in Turkey covers an area of 5.444 ha. Elevation in the area ranges from 40 to 230 meters. The climate of Belgrad Forest according to Thornthwaite classification system is humid, mesothermal oceanic with a moderate water deficit in summer. The soils are shallow to deep, gravelly, loamy clay in texture, rich in organic matter with medium to good permeability rates. Dominant vegetation includes *Quercus frainetto*, *Q. cerris* and *Fagus orientalis* tree species mixed with varying amounts of *Acer campestre*, *A. trautvetteri*, *Alnus glutinosa*, *Carpinus betulus*, *Castanea sativa*, *Populus tremula*, *Sorbus torminalis* and *Ulmus campestris* with a normal crown closure (Yaltirik, 1966; Kantarci, 1980; Karaoz, 1988; Ozyuvaci *et. al.*, 2004).

The name of horse chestnut is given for its speciality of being used against heaves (Chronic Obstructive Pulmonary Disease) after completing of the horse racing. This tree species with a domed crown grows up to about height of 25 m. The outer branches of the old trees lean out of during vegetation period because of heights of leaves. During winter season branches falling leaves turn towards up again. The leaves are opposite and palmately compound, with leaflets; each leaflet is 10-25 cm long, making the whole leaf up to 50 cm across, with a 20 cm petiole (Anonymous, 2006b; Anonymous, 2007). *A. hippocastanum* is an exotic plant species for Turkey and is widely cultivated in Belgrad Forest. *A. hippocastanum* is generally used for parks and gardens or afforestation of gaps because of the fast and good growing on each area such as native species. It offers an aesthetic image and protection against the noises, dusts, air pollutions and heat in urban areas of Turkey. The horse chestnut is also an ornamental plant species used in Landscape architecture planning. But, there is almost no information about its pests in Turkey. Although some insects were observed feeding on *A. hippocastanum* but more of this studies investigated directly Lepidopterous species that cause problems on this tree species. On the other hand, in European and Balkan countries it is especially well known the *A. hippocastanum* is severely damaged by some insects (Ariëns, 2004; Augustin *et. al.*, 2004; Avtziş, 2004; Baraniak *et. al.*, 2004; Del Bene / Gargani, 2004; Gininenko, 2004; Girardoz *et. al.*, 2004; Johné *et. al.*, 2006; Matošević, 2004; Matošević *et. al.*, 2006; Milevoj, 2004; Milevoj / Pivk, 2004; Perju *et. al.*, 2004; Subchev *et. al.*, 2004; Tilbury *et. al.*, 2004). In contrast, nobody investigated insect problems on this tree species in Turkey. Therefore, the aim of this study was to investigate harmful Lepidopterous species causing disease on horse chestnut adapting itself the climate conditions of Turkey.

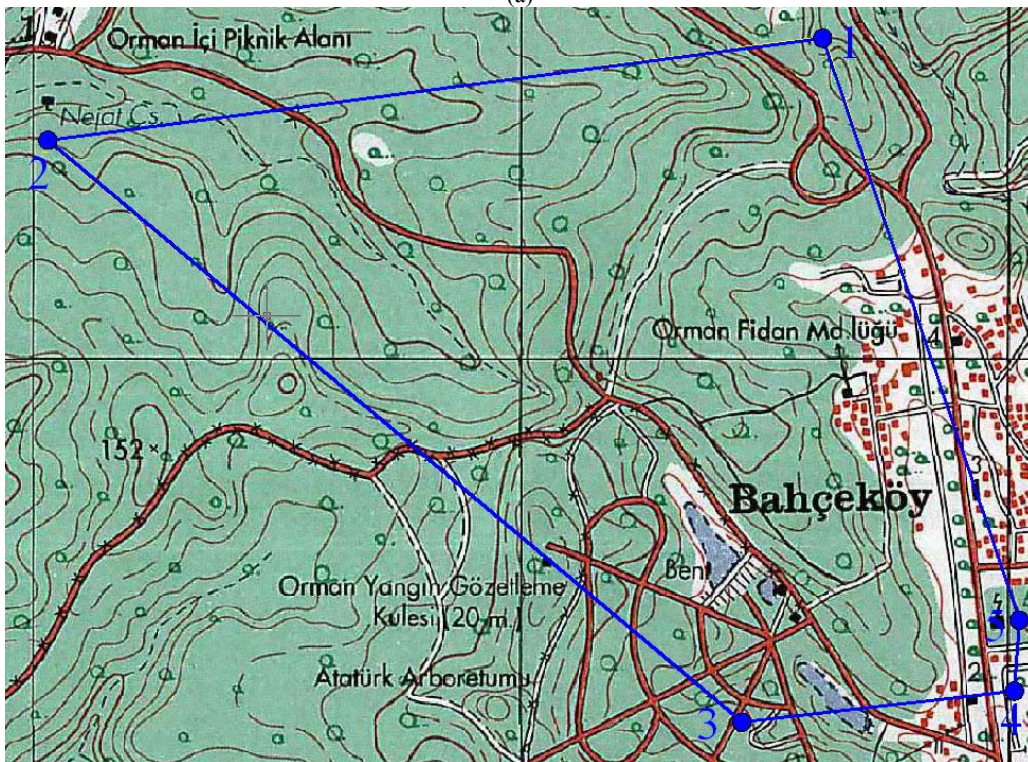
MATERIAL AND METHODS

This study was conducted between 2004 and 2005 years in Istanbul-Belgrad Forest. The horse chestnut trees are distributed on sites (1, 2, 3, 4 and 5) in forest area (Figure 1). The horse chestnut trees were monitored for the presence of pests. Larvae on infested leaves, parts of trunks and shoots were picked up from April to October. The pests were fed regularly inside glass bottles covered organandy mesh in the laboratory. Also, *Cameraria ohridella* was monitored into bags hanging on tree branches. Relative humidity in the laboratory was maintained 60-70% within 20-24 °C. The emerged adults were killed in killing jars with ethyl acetate. Each specimen was pinned using a no: 1 insect pin and the wings were mounted on

a spreading board. After, the dried specimens were numbered and placed in insect boxes. Each Lepidopteran specimen was identified using our collections by an Olympus stereomicroscope.



(a)



(b)

Figure 1. (a) The map of Belgrad Forest.
 (b) Sites of founded chestnut trees in forest area (1, 2, 3, 4 and 5)

RESULTS

Lepidopteran species determined belong to four families Gracillariidae, Tortricidae, Geometridae and Noctuidae, and are shown below:

Cameraria ohridella (Deschka & Dimic, 1986) (Gracillariidae)

04.vi.2004 (larva), 06.ix.2004 (pupa), 27.ix.2004, 1 ♂; 04.vii.2004 (larva), 10.ix.2004 (pupa), 01.x.2004 1 ♂; 11.viii.2004 (larva), 15.ix.2004 (pupa), 04.x.2004, 1 ♀; 11.viii.2004 (larva), 12.ix.2004 (pupa), 02.x.2004, 1 ♀; 12.ix.2004 (larva), 17.ix.2004 (pupa), 13.x.2004, 1 ♀; 11.viii.2005 (larva), 04.ix.2005 (pupa), 24.ix.2005, 1 ♀; 11.viii.2005 (larva), 05.ix.2005 (pupa), 30.ix.2005, 1 ♂; 06.ix.2005 (larva), 10.ix.2005 (pupa), 03.x.2005, 1 ♂.

The moth was more abundantly in the study field. It was founded 3-9 larvae in point of numbers on each leaf, generally 6-9 larvae on lower branches and 3-5 larvae mid branches.

Archips crataegana (Hübner, 1799) (Tortricidae)

Istanbul-Bebek, Belgrad Forest on *Crataegus monogyna* and *Quercus robur* (De Lattin, 1951; Avci, 1997; Kocak / Seven, 2001a).

04.v.2005 (larva), 13.v.2005 (pupa), 30.v.2005, 1 ♂.

Archips podana (Scopoli, 1763) (Tortricidae)

Adana, Bursa, Canakkale, Istanbul, Izmit, Kirklareli on *Cornus mas*, *Corylus colurna*, *Fraxinus angustifolia*, *Malus* sp., *Populus* sp., *Quercus frainetto*, *Rosa* sp. and *Tilia argentea* (Osthelder, 1935; Bodenheimer, 1941; De Lattin, 1951; Yigit / Uygun, 1982; Avci, 1997; Kocak, / Seven, 2001a; Ozbek / Calmasur, 2005).

03.v.2004 (larva), 25.v.2004 (pupa), 07.vi.2004, 1 ♂; 05.v.2004 (larva), 18.v.2004 (pupa), 05.vi.2004, 1 ♀; 11.v.2005 (larva), 02.vi.2005 (pupa), 09.vi.2005, 1 ♂.

Archips xylosteana (Linnaeus, 1758) (Tortricidae)

Bartin, Bursa-Mustafakemalpasa, Istanbul-Alemdag, Belgrad Forest, Florya, Yildiz Park, Izmir-(Bornova, Torbali), Kirklareli-Kofcaz, Kocaeli on *Acer campestre*, *Ailanthus* sp., *Carpinus betulus*, *Cercis siliquastrum*, *Corylus colurna*, *Fraxinus* sp., *F. ornus*, *Lonicera* sp., *Pistacia* sp., *Platanus* sp., *Populus tremula*, *Rubus* sp., *Quercus* sp., *Q. petraea* subsp. *iberica*, *Q. robur*, *Rhododendron* sp., *Rosa* sp., *Salix* sp., *Tilia argentea*, *Ulmus* sp. (Bodenheimer, 1941; Acatay, 1943; Aysu, 1951; Alkan, 1962; Sekendiz, 1974; Canakcioglu, 1982; Uzun / Yalcin, 1992; Avci, 1997; Kocak / Seven, 2001a; Ozbek / Calmasur, 2005).

30.iv.2004 (larva), 18.v.2004 (pupa), 06.vi.2004 1 ♂; 5.v.2004 (larva), 17.v.2004 (pupa), 31.v.2004 1 ♀; 11.v.2004 (larva), 18.v.2004 (pupa), 10.vi.2004 1 ♀; 10.v.2005 (larva), 17.v.2005 (pupa), 30.v.2005 1 ♀.

Crocallis elinguaris (Linnaeus, 1758) (Geometridae)

Usak-Akse plantation (Mol, 1977; Kocak / Seven, 2001b).

11.v.2005 (larva), 18.v.2005 (pupa), 20.vi.2005 1 ♂.

Ennomos quercaria ([Hübner], [1812]) (Geometridae)

Istanbul-Belgrad Forest, Kirklareli-Demirkoy, Aegean region on *Aesculus hippocastanum*, *Carpinus betulus*, *Fagus orientalis*, *Quercus petraea* subsp. *iberica*, *Q. pubescens*, *Q. robur* and *Salix* sp. (Mol, 1977; Kocak / Seven, 2001b).

25.iv.2004 (larva), 28.v.2004 (pupa), 07.vi.2004, 1 ♂; 03.v.2004 (larva), 30.v.2004 (pupa), 14.vi.2004, 1 ♀.

Ennomos quercinaria (Hufnagel, 1767) (Geometridae)

Bursa, Istanbul-Sariyer, Kirklareli-Demirkoy, Kirsehir on *Quercus* sp. (Staudinger, 1881; Kansu, 1963; Okyar / Aktac, 1999).

05.v.2004 (larva), 30.v.2004 (pupa), 11.vi.2004, 1 ♀; 05.v.2004 (larva), 31.v.2004 (pupa), 10.vi.2004 1 ♀.

Erannis defoliaria (Clerck, 1759) (Geometridae)

Bursa-Karabelen, Edirne-Tavuk Forest, Istanbul-Belgrad Forest, Kahramanmaras, Kirklareli-Demirkoy, Samsun-Gelemen, Trabzon-Meryemana on *Acer platanoides*, *Alnus glutinosa*, *Betula* sp., *Carpinus betulus*, *Castanea sativa*, *Corylus colurna*, *Fagus orientalis*, *Quercus robur*, *Rosa* sp., *Salix alba*, *Sorbus torminalis*, *Tilia* sp. and *Ulmus* sp. (Osthelder, 1935; Mol, 1977; Ozay, 1997; Yuksel, 1998; Okyar / Aktac, 1999; Kocak / Seven, 2001b).

05.v.2004 (larva), 17.v.2004 (pupa), 28.xii.2004, 1 ♀; 05.v.2004 (larva), 18.v.2004 (pupa), 03.i.2005, 1 ♂; 05.v.2004 (larva), 18.v.2004 (pupa), 17.i.2005, 1 ♂; 11.v.2004 (larva), 19.v.2004 (pupa), 06.i.2005, 1 ♂; 04.v.2005 (larva), 17.v.2005 (pupa), 11.i.2006, 1 ♂.

Operophtera brumata (Linnaeus, 1758) (Geometridae)

Canakkale-Koru Mountain, Edirne-Tavuk Forest, Istanbul-Belgrad Forest, Kirklareli-Demirkoy-Igneada, Aegean region on *Acer* sp., *Carpinus betulus*, *Castanea sativa*, *Corylus colurna*, *Populus* sp., *Quercus* sp., *Q. petraea* subsp. *iberica*, *Q. robur*, *Rhododendron* sp., *Salix* sp., *S. alba*, *Ulmus* sp. (Mol, 1977; Ozay, 1997; Okyar / Aktac, 1999; Kocak / Seven, 2001b).

03.v.2004 (larva), 20.v.2004 (pupa), 01.i.2005, 1 ♂; 03.v.2004 (larva), 20.v.2004 (pupa), 24.i.2005, 1 ♀; 03.v.2004 (larva), 20.v.2004 (pupa), 03.i.2005, 1 ♂; 03.v.2004 (larva), 20.v.2004 (pupa), 18.i.2005, 1 ♂; 04.v.2005 (larva), 17.v.2005 (pupa), 30.xii.2005, 1 ♀; 04.v.2005 (larva), 31.v.2005 (pupa), 14.i.2006, 1 ♀; 04.v.2005 (larva), 30.v.2005 (pupa), 17.i.2006, 1 ♂; 10.v.2005 (larva), 29.v.2005 (pupa); 13.i.2006, 1 ♂.

Pachynemina hippocastanaria (Hübner, 1799) (Geometridae)

Amasya, Bursa, Canakkale-Ecebat (Staudinger, 1881; Okyar / Aktac, 1999; Kocak / Seven, 2001b).

30.iv.2004 (larva), 20.v.2004 (pupa), 18.i.2005, 1 ♂; 04.v.2005 (larva), 16.v.2005 (pupa), 22.i.2006, 1 ♂.

Amphipyra pyramidea (Linnaeus, 1758) (Noctuidae)

Duzce, Istanbul-Belgrad Forest, Tekirdag-Malkara, Zonguldak-Centre and Eregli on *Carpinus betulus*, *Fagus orientalis*, *Quercus* sp. (De Lattin, 1951; Hacker, 1987; Hakyemez, 1994; Kocak / Seven, 2001b; Akbulut *et. al.*, 2003).

14.v.2004 (larva), 18.v.2004 (pupa), 17.vi.2004, 1 ♂.

DISCUSSION AND CONCLUSIONS

Most of obtained species are polyphagous and these species prefer forest trees than ornamentals.

Cameraria ohridella was the most important dominant insect species found on horse chestnuts in this study. However, this situation does not cause vitality problems on trees at least for now. The information about this species recorded in previous studies was not encountered during this study. *Cameraria ohridella* was not reported in the studies carried out previously in Turkey whereas it was the most dominant species found on chestnut trees in Belgrad Forest. Although *Cameraria ohridella* is well

known species in European and Balkan countries due to its damages on *A. hippocastanum* there is no information about this insect in Turkey.

Among the collected polyphagous species, *Archips crataegana*, *A. podana*, *A. xylosteana*, *Crocallis elinguaris*, *Erannis defoliaria*, *Operophtera brumata*, *Amphipyra pyramidea* and the monophagous species on *Quercus* sp., *Ennomos quercinaria*, were carried out on *Aesculus hippocastanum* in Turkey by this study. The obtained findings related to *Ennomos quercaria* living on *A. hippocastanum* in the Belgrad Forest are harmony with the recorded literature by Mol.

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Agrovoc descriptors: meloidogyne javanica; nematoda; lycopersicon esculentum; greenhouses; grafting; scions; infection

Agris category code: H10

COBISS code 1.01

A case of infection on the scion of grafted tomatoes by the root-knot nematode *Meloidogyne javanica*

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ABSTRACT

This is a preliminary communication reporting on a case where the scion of a grafted tomato in a greenhouse crop of Crete became infected by the root-knot nematode *Meloidogyne javanica*.

Key words: greenhouse crop, grafting, nematodes

IZVLEČEK

PRIMER OKUŽBE CEPLJENEGA PARADIŽNIKA Z NEMATODO *Meloidogyne javanica*

Predhodno poročilo o primeru, da je cepič cepljenega paradižnika v rastlinjaku na Kreti okužila nematoda *Meloidogyne javanica*.

Ključne besede: vrtnina v rastlinjaku, cepljenje, nematode

1 INTRODUCTION

Grafting vegetables on resistant rootstocks is a mean of controlling root-knot nematodes in areas with intensive land use (Lee, 1994; Greco, 1999; Ioannou, 2001; Kacjan Maršić and Osvald, 2004; Lopez-Perez et al., 2006).

Despite several grown tomatoes are resistant against the root-knot nematode species *Meloidogyne javanica*, *M. incognita* and *M. arenaria* (Williamson, 1998), the desirable fruit characteristics are not always available in the nematode resistant cultivars. In these cases susceptible varieties with the commercially required characteristics can be grafted onto nematode resistant rootstocks. In Greece the interest in growing grafted plants to control root-knot nematodes, especially in greenhouses, has been increased, but published data are available only for cucumber (Giannakou and Karpouzas, 2003).

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An investigation of a problem observed in a grafted greenhouse tomato crop is described below.

2 MATERIALS AND METHODS

In a greenhouse of Crete with known problem of root-knot nematodes, a tomato crop had been established with a susceptible cv. grafted on a resistant rootstock. That was an evaluation of experimental genotypes under the development to become cultivars by a Seed Company. Many plants remained stunted and chlorotic and produced small fruits. Root samples were brought in the lab and examined.

3 RESULTS AND DISCUSSION

Several galls were observed on roots deriving from the scion. In contrast, the rootstock was free of galls but small in size (Figure 1). The galls of the scion root were dissected and revealed the presence of several females and egg masses of root-knot nematodes. The population was identified as *M. javanica*.

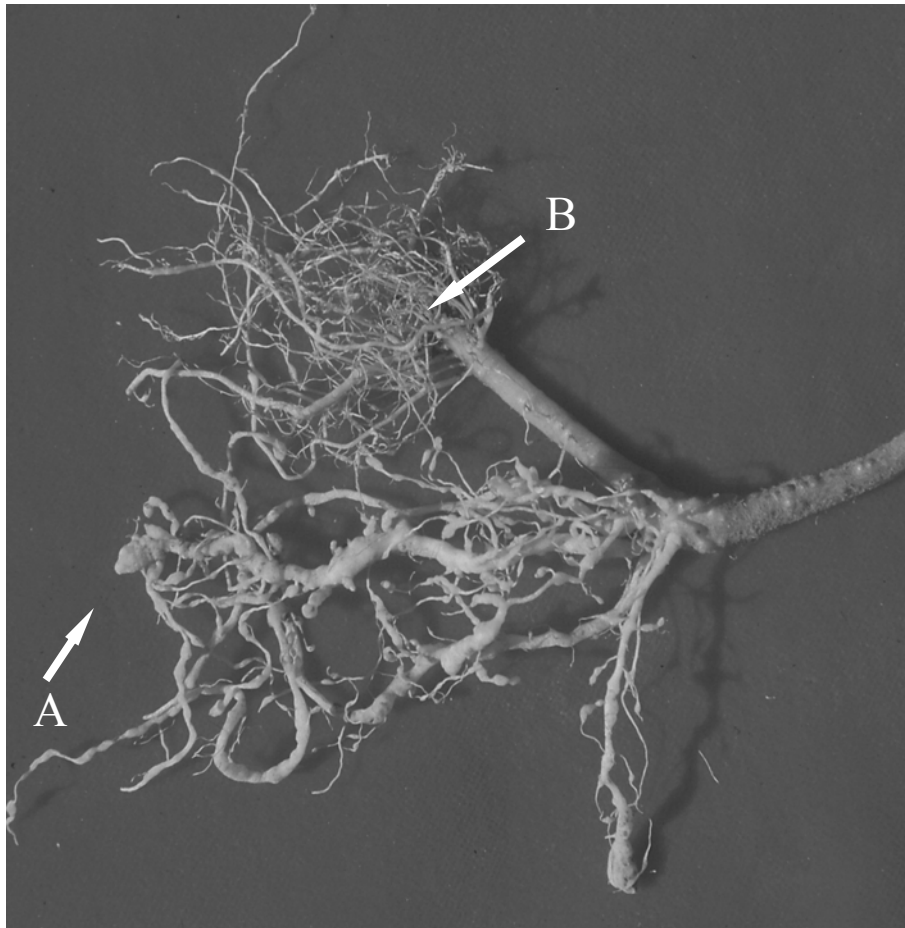


Figure 1. Root galls of *M. javanica* on a root produced by a scion (A) in a grafted tomato plant. The resistant rootstock (B) remained uninfected.

The certain plants had been planted deeply and the point of grafting was very close to the soil surface or had been covered with soil. The great humidity caused rooting of the

scion and this newly produced root became larger than that of the rootstock and as was susceptible, became infected by nematodes. It is hypothesized that the plant was receiving the majority of water and nutrients from the scion's root system and that explains the appearance of symptoms typical of nematode infection. These problems of scion rooting should be avoided at planting taking care so as the grafting point not to be close to soil surface. Furthermore cultivation techniques should prevent the formation of soil piles around the plant stem.

The use of grafted tomatoes on resistant rootstocks as a mean of managing nematode populations should be investigated in greenhouse crops of Greece. Rotation of resistant tomato with susceptible has been already proved successful for reducing *M. javanica* infestations in greenhouse conditions of Crete (Tzortzakakis et al., 2000). Further research is also required in accessing the susceptibility of tomato rootstocks to resistance breaking pathotypes of *Meloidogyne*, which have been found infecting resistant tomatoes in both field and experimental conditions (Tzortzakakis et al., 1999, 2000, 2005).

4 ACKNOWLEDGMENTS

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Agris category code: F01, F62, F61

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Response of onion (*Allium cepa* L.) to combined application of biological and chemical nitrogenous fertilizers

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ABSTRACT

A field experiment was conducted during the summer season of 2000-2001 at the Vegetable Research Farm of Indian Agricultural Research Institute (I.A.R.I.), New Delhi (India) to test the efficacy of three *Azotobacter* strains as a potential supplement to nitrogenous fertilizer in improving growth and yield of onion cv. Pusa Madhvi. The treatments consisted of factorial combination of four levels of nitrogen (0, 25, 50 and 75 kg N ha⁻¹) and three *Azotobacter* strains (CBD-15, AS-4 and M-4) with two uninoculated controls one with full dose of N (100 kg ha⁻¹) and the other without NPK. Application of 75 kg N ha⁻¹ along with inoculation of CBD-15 was found to have significantly increased most of the growth and yield parameters, soil available nitrogen, and nitrogen content in the bulb followed by M-4 inoculation as compared to application of full dose of nitrogen without the inoculation. Days to bulb initiation were significantly reduced due to inoculation with CBD-15 or M-4 along with 50 kg N ha⁻¹ whereas days to bulb maturity were significantly reduced due to inoculation with any of the strains along with the same N rate (50 kg ha⁻¹) as compared to application of full dose of nitrogen without the inoculation. The finding demonstrated a saving of 50 kg N ha⁻¹ without significantly affecting yield and an average increase of 13.5% marketable yield due to *Azotobacter* inoculation in the presence of 75 kg N ha⁻¹.

Key words: *Azotobacter*, onion, nitrogenous fertilizer, growth, yield, available nitrogen, bulb diameter

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IZVLEČEK

VPLIV KOMBINIRANE APLIKACIJE *Azotobacter*-JA IN DUŠIČNEGA GNOJILA NA PRIDELEK ČEBULE (*Allium cepa* L.)

V letih 2000-2001 je bil na Vegetable Research Farm, Indian Agricultural Research Institute (I.A.R.I.), New Delhi (India) izveden poskus za preverjanje učinkovitost treh sojev *Azotobacter* kot možnih dodatkov k uporabi dušičnih gnojil, da bi se izboljšalo rast in pridelek pri čebuli cv. Pusa Madhvi. Poskus je vključeval faktorske kombinacije štirih ravni preskrbljenosti z dušikom (0, 25, 50 and 75 kg N ha⁻¹) in tri genotipe *Azotobacter* (CBD-15, AS-4 and M-4), z dvema neinokuliranimi kontrolama, od tega eno z odmerkom N (100 kg ha⁻¹) in drugo brez gnojenja z NPK. Aplikacija 75 kg N ha⁻¹ je skupaj z inokulacijo CBD-15 dala pomembno povečanje večine parametrov rasti in pridelka, v tleh dostopnega dušika in vsebosti dušika v čebulah po inokulaciji z M-4, v primerjavi z aplikacijo polnega odmerka dušika a brez inokulacije. Število dni do začetka tvorbe čebul je bilo značilno manjše pri inokulaciji z CBD-15 ali M-4, skupaj z odmerkom 50 kg N ha⁻¹. Število dni do zrelih čebul je bilo značilno manjše pri inokulaciji s katerim koli genotipom pri enakem odmerku N (50 kg ha⁻¹), v primerjavi s polnim odmerkom dušika brez inokulacije. Ugotovitev kažejo prihranek 50 kg N ha⁻¹ brez značilnega znižanja pridelka. Ugotovljeno je povprečno povečanje tržnega pridelka za 13,5% pri inokulaciji z *Azotobacter*-jem in odmerku 75 kg N ha⁻¹.

Ključne besede: *Azotobacter*, čebula, dušično gnojilo, rast, pridelek, dostopen dušik, premer čebul

INTRODUCTION

Onion (*Allium cepa*) is one of the most important vegetable crops grown and used throughout the world. Onion being among the high nitrogen demanding vegetables, its productivity depends on use of optimum fertilizer rates and if not adequately fertilized, considerable yield losses are apparent. The present day modern agriculture depends heavily on use of chemical fertilizer for boosting crop yield. However, these fertilizers are often in short supply and their indiscriminate use has an adverse effect on long-term soil health and environment, which has received global attention. Moreover, chemical fertilizers are costly and hence are hardly affordable by small and marginal farmers, which constitute the majority of the farming community in developing countries. The most realistic solution is, therefore, to exploit the possibility of supplementing chemical fertilizers with organic ones, more particularly biofertilizers of biological origin. These days biofertilizers have emerged as an important component of integrated nutrient management strategy and had a promise to improve an over all crop performance, yield and nutrient supply. Thus, of late increasing attention is being paid to derive the most benefit from biofertilizers. Biofertilizers are low cost, effective and renewable source of plant nutrients to supplement chemical fertilizers and their role in onion as well as other vegetable production, therefore, assumes a special significance, particularly in the present context of very high cost of chemical fertilizers.

Onion responds well to *Azotobacter*ization and yield increase up to 20 per cent has been reported (Meshram and Shende, 1990). However, there exists wide variation in nitrogen fixing capacity of various strains of *Azotobacter* (Vinay,

1998) and strain specificity to crop plants has also been reported (Rajakumar and Lakshman, 1990). The present study was, therefore, conducted with the view to identify efficient strain of *Azotobacter* and to select a suitable N level and *Azotobacter* strain combination(s) for better growth and yield of onion.

MATERIALS AND METHODS

A field experiment was conducted using onion cultivar Pusa Madhvi during the summer season of 2000-2001 at the Vegetable Research Farm of Indian Agricultural Research Institute (IARI), New Delhi, on sandy loam soil having a pH of 7.9. Four levels of nitrogen (0, 25, 50 and 75 kg N ha⁻¹) and three *Azotobacter* strains (CBD-15, AS-4 and M-4) were combined factorially with two additional uninoculated controls one with full dose of N, 100 kg N ha⁻¹, (standard practice) and the other without NPK (absolute control). A total of 14 treatments including controls were laid out in Randomized Block Design (RBD) with three replications. A plot size of 4.68 m² and spacing of 10cm x 15cm were used. A carrier based (Charcoal: Soil, 3:1) inoculum of each of the *Azotobacter* strain @ 500 g ha⁻¹ was suspended in water to prepare a slurry and seedlings were uprooted from the nursery beds and dipped in the respective *Azotobacter* strain slurry for transplanting in the main field. Only half dose of nitrogen as urea was applied at the time of transplanting and the rest half was applied in two equal splits at 30 and 50 days after transplanting. Full doses of phosphorus as single super phosphate and potassium as muriate of potash were applied to all the treatments at transplanting @ 50 kg P₂O₅ and 75 kg K₂O ha⁻¹, respectively. Data was recorded on growth parameters such as plant height, number of leaves per plant both at 45 and 90 days after planting. Observation was also made on days to bulb initiation, days to maturity, bulb weight, bulb diameter, total and marketable yields, bulb nitrogen content and soil available nitrogen.

RESULTS AND DISCUSSION

1. Plant height and number of leaves per plant

At 45 days after transplanting (DAT), only plant height was significantly influenced due to *Azotobacter* inoculation under all levels of nitrogen tested when compared to the control with 100 kg N ha⁻¹ (Table 1). However, at 90 DAT, both plant height and number of leaves per plant was significantly influenced due to nitrogen amendment with *Azotobacter* inoculation. Application of 75 kg N ha⁻¹ in combination with CBD-15 or M-4 significantly improved all parameters over the control treatment with 100 kg N ha⁻¹. Such increase in plant height due to *Azotobacter* inoculation have also been reported by (Badaway and Amer, 1974; Martinez *et al.*, 1994) in tomato and (Dibut *et al.*, 1993; Rita, 1993 and Mandhare *et al.*, 1998) in onion.

2. Days to bulb initiation and maturity

Days to bulb initiation and maturity showed variations both among the nitrogen levels and *Azotobacter* strains. In the absence of nitrogen, inoculation of *Azotobacter* did not significantly reduce the duration of bulb initiation and maturity. Similarly, inoculation of *Azotobacter* strain in the presence of 25 and 75 kg N ha⁻¹ resulted in days to bulb initiation and maturity that was statistically at par with both uninoculated controls. However, application of 50 kg N ha⁻¹ along with the inoculation of CBD-15 or M-4 resulted in 4 and 3.7

days earlier bulb initiation, respectively over the standard control. Likewise, application of the same N level (50 kg N ha⁻¹) along with the inoculation of any of the *Azotobacter* strains led to 1.7 to 2.7 days earlier bulb maturity as compared to the full dose of nitrogen (100 kg N ha⁻¹) application without the inoculation. Similarly, early bulb initiation and maturity in onion was reported by Rita (1998) due to *Azotobacter* inoculation. The earliness in bulb initiation and maturity may be attributed to the ability of the bacterium to produce growth promoting substances which might have induced bulbing at earlier stage and there by enhanced chance of early crop maturity. Further more, inoculation along with high dose of nitrogen could not be as effective as inoculation along with moderate or lower doses in reducing days to maturity due to prolonged vegetative growth period in the former case.

3. Bulb diameter and 10 bulb weight

An overall increase of 11.2% and 8.5% higher horizontal and vertical bulb diameter, respectively over the control treatment with 100 kg N ha⁻¹ were obtained due to inoculation of CBD-15 along with 75 kg N ha⁻¹ and it was closely followed by the treatment with 75 kg N ha⁻¹ + M-4 (Table 1). The values for both treatments were statistically significant when compared to the control treatment with 100 kg N ha⁻¹. Similar trend was observed for weight of 10 bulbs. Amendment of 75 kg N ha⁻¹ through inoculation of either CBD-15 or M-4 resulted in 15 and 12.5 % increased weight of 10 bulbs, respectively over the control treatment with 100 kg N ha⁻¹ (Table 1).

4. Total and marketable yields

Supplementation of nitrogen fertilizer with *Azotobacter* inoculation markedly increased both total and marketable yields. Supplementing 75 kg N ha⁻¹ through inoculation of either CBD-15 or M-4 gave an increase of 12.9 and 9.9% in total yield; and 15 and 11.9%, respectively in marketable yields over the uninoculated control with 100 kg N ha⁻¹. The result also clearly demonstrate that inoculation of CBD-15 or M-4 in the presence of 50 kg N ha⁻¹ gave yield statistically at par with the uninoculated control having full dose of N (100 kg N ha⁻¹) there by resulting in a net saving of 50 kg N ha⁻¹. Similarly, (Konde *et al.*, 1978 and Rita, 1998) have also reported increased total yield in onion due to *Azotobacter* inoculation. Bhonde *et al.* (1997) reported the highest marketable yield (230.62 q ha⁻¹) in onion due to combined application of *Azotobacter* and 50% recommended dose of nitrogenous fertilizer.

5. Soil available nitrogen and bulb nitrogen content

A remarkable increase in soil available nitrogen after crop harvest was observed due to supplementation of 75 kg N ha⁻¹ with inoculation of CBD-15 over the control with full dose of nitrogen as well as over the absolute control by giving 23.6 kg ha⁻¹ and 104.5 kg ha⁻¹ higher soil available nitrogen over both the controls, respectively (Table 2). Inoculation with all the strains except in the

absence of applied nitrogen, have furnished the soil with additional quantity of available nitrogen compared to the amount that was present before the crop was planted (236.37 kg ha⁻¹). Likewise these findings draw support from several other earlier reports. Ahmad (1998) reported the highest soil available nitrogen due to application of 145g N tree⁻¹ + *Azotobacter* strain CBD-15 over 145g N tree⁻¹ + *Azotobacter* strain M-4 and all other treatments which is in close conformity with the present finding. Nitrogen content in bulbs was significantly increased due to supplementation of 75 kg N ha⁻¹ with either CBD-15 or M-4 over the control with full dose of nitrogen (Table 2). These two treatments gave 0.24 and 0.16 per cent higher nitrogen in bulbs over the standard practice and 1.17 and 1.0 per cent higher nitrogen in bulbs over the absolute control, respectively. A significant increase in percent nitrogen in grain as well as stover of maize was also reported due to *Azotobacter* inoculation along with moderate amount of nitrogenous fertilizer by Meshram and Shende (1982). The increase in nitrogen content in bulbs might be due to better root development that was achieved as a result of inoculation with efficient strains, which led to enhanced nutrient uptake.

CONCLUSION

Of the three strains of *Azotobacter* used, two strains (CBD-15 and M-4) in the presence of 75 kg N ha⁻¹ were found to have significantly improved the growth and yield parameters, nitrogen content in the bulb and soil available nitrogen when compared to uninoculated control with 100 kg N ha⁻¹. An average increase of 13.5% in marketable yield was achieved due to effective *Azotobacter* strain inoculation in the presence of 75 kg N ha⁻¹ over uninoculated control with 100 kg N ha⁻¹. Inoculation with the effective strains (CBD-15 and M4) also enabled a saving of 50 kg N ha⁻¹ with out affecting total and marketable yields as compared to the uninoculated control treatment with 100 kg N ha⁻¹. The increase in growth and yield parameters in the inoculated treatments can be attributed to the multiple effects of *Azotobacter* such as their ability to fix atmospheric nitrogen (Pandey *et al.*, 1989) suppression of pathogenic micro-organisms (Lakshmi Kumari *et al.*, 1972; Meshram and Jagar, 1983), production of growth promoting substances (Brown, 1974, Shende *et al.*, 1975). Moreover, its role in solubilization of phosphate (Iswaran and Marwaha, 1981) and general improvement in nutrient uptake of the plant due to root proliferation might have also considerably contributed to enhanced growth and yields of the inoculated treatments in these findings.

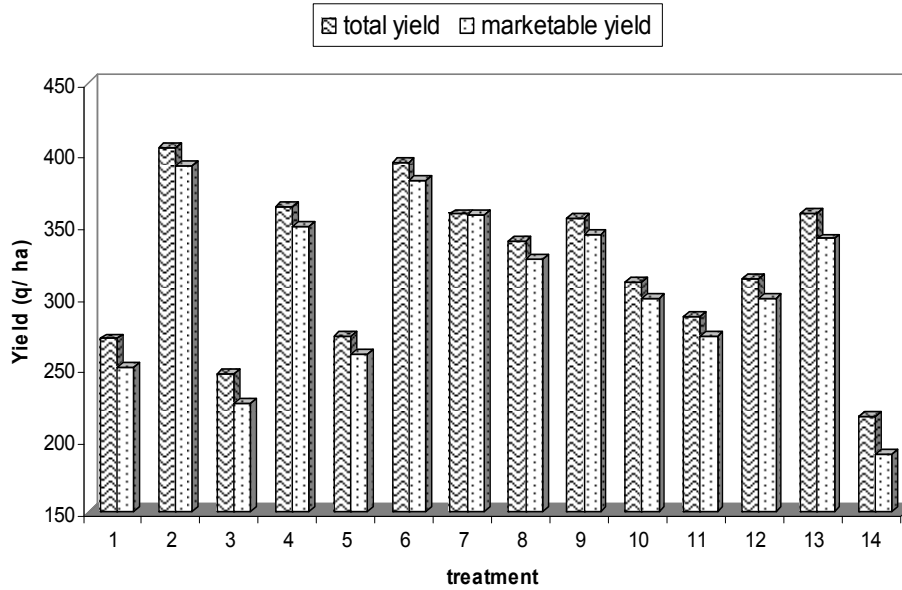


Figure 1. Total and marketable yields of onion as affected by combined application of *Azotobacter* strains and reduced N doses

Table 1. Various growth parameters of onion as affected by combined application of *Azotobacter* strains and reduced N doses

Treat.	Plant height (cm)		No. of leaves per plant		Days to bulb initiation	Days to maturity	Bulb diameter (cm)		10 bulb weight (g)	
	45 DAT	90 DAT	45 DAT	90 DAT			Vertical	Horizontal	Fresh	Dry
T1	30.27	47.87	4.20	7.03	73.33	128.7	4.10	5.33	656.7	56.40
T2	39.63	59.63	4.87	8.67	70.33	127.0	5.67	6.80	920.0	97.40
T3	29.80	47.00	4.07	6.80	73.67	129.0	4.00	5.17	640.0	53.30
T4	36.00	54.43	4.80	8.13	71.00	127.3	5.23	6.17	880.0	89.00
T5	30.93	49.00	4.32	7.17	73.00	128.3	4.23	5.33	673.3	59.23
T6	39.40	58.87	4.87	8.53	70.67	127.0	5.60	6.73	900.0	96.83
T7	35.90	55.23	4.67	7.99	68.00	125.0	5.00	6.23	876.7	86.33
T8	34.53	53.67	4.55	7.50	69.33	126.0	4.87	6.03	836.7	81.37
T9	34.77	54.33	4.60	7.97	68.33	125.3	5.03	6.10	860.0	83.20
T10	32.60	51.03	4.50	7.60	71.67	126.3	4.60	5.63	750.0	69.63
T11	31.53	50.17	4.40	7.33	72.33	126.7	4.47	5.50	740.0	67.63
T12	33.30	52.13	4.53	7.60	71.33	126.0	4.70	5.73	770.0	73.30
T13	36.07	54.00	4.73	8.00	72.00	127.7	5.10	6.27	880.0	83.13
T14	27.67	44.17	3.39	6.13	74.00	130.0	3.73	4.37	560.0	49.07
C.D. (5%)	3.20	4.10	0.39	0.51	3.35	1.52	0.42	0.45	37.40	13.57
T ₁ = 0 kg N ha ⁻¹ + CBD-15	T ₂ = 75 kg N ha ⁻¹ + CBD-15		T ₃ = 0 kg N ha ⁻¹ + AS-4							
T ₄ = 75 kg N ha ⁻¹ + AS-4	T ₅ = 0 kg N ha ⁻¹ + M-4		T ₆ = 75 kg N ha ⁻¹ + M-4							
T ₇ = 50 kg N ha ⁻¹ + CBD-15	T ₈ = 50 kg N ha ⁻¹ + AS-4		T ₉ = 50 kg N ha ⁻¹ + M-4							
T ₁₀ = 25 kg N ha ⁻¹ + CBD-15	T ₁₁ = 25 kg N ha ⁻¹ + AS-4		T ₁₂ = 25 kg N ha ⁻¹ + M-4							
T ₁₃ = Full dose of N (100 kg N ha ⁻¹) without <i>Azotobacter</i> (standard practice)	T ₁₄ = Without NPK and <i>Azotobacter</i> (absolute control)									

Table 2: Soil available nitrogen and bulb nitrogen content as affected by combined application of *Azotobacter* strains and reduced nitrogen doses

Treatment	Available nitrogen in soil (kg ha ⁻¹)	Nitrogen content (%) in Bulbs
T ₁	220.5	2.53
T ₂	295.5	3.57
T ₃	214.5	2.50
T ₄	262.9	3.35
T ₅	224.2	2.60
T ₆	290.6	3.49
T ₇	269.7	3.22
T ₈	254.5	3.16
T ₉	267.6	3.21
T ₁₀	245.3	2.89
T ₁₁	241.0	2.79
T ₁₂	249.5	2.91
T ₁₃	271.9	3.33
T ₁₄	191.0	2.40
C.D. (at 5%)	23.40	0.12

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Agrovoc descriptors: rural areas; tourism; natural resources; land resources; environmental protection; national parks; nature reserves; sociocultural environment; sociocultural environment; cultural values; value systems

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Rural tourism and protected areas – factors to increase resilience of rural areas¹

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ABSTRACT

The study analysed the influence of presence of protected area on the vulnerability and resilience of the surrounding region in different socio-economic and natural shocks and perturbations. For the study two areas were selected: area of Triglav national park (TNP), as area with highly diversified rural tourism and area of Kozjansko regional park (KRP) as area with low level of rural tourism diversification. The primary data collection was conducted with use of in-depth interviews among relevant stakeholders. In each area some interviews were carried out, where some interviews were representing multiple stakeholders also. The results of the analysis mostly confirmed our hypothesis. We could recognize that the diversity of actors and social roles are essential as sources of stability, resilience, robustness and integrity in the social dimension of natural resource management, that an ecological regime shift or collapse does not necessarily result in a regime-shift or collapse of the social-ecological system, that an adaptive governance framework relies critically on the collaboration of a diverse set of stakeholders operating at different social and ecological scales, that good governance of the socio-economic domain does not necessarily imply maintaining a stable / resilient / robust / integer social-ecological system and that institutions, social networks and organisation interact across scales. On the other hand a hypothesis that more diverse tourism leads to higher stability, resilience, robustness and integrity of social-ecological systems was not confirmed.

Key words: rural tourism, protected areas, resilience, socio-ecological system, rural development, innovation, learning, vulnerability

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IZVLEČEK

PODEŽELSKI TURIZEM IN ZAVAROVANA OBMOČJA – DEJAVNIKI POVEČANJA PROŽNOSTI PODEŽELSKIH OBMOČIJ

V raziskavi je bil preučevan vpliv prisotnosti zavarovanega območja na ranljivost in prožnost širše regije ob različnih socio-ekonomskih in naravnih šokih in motnjah. Raziskava je bila izpeljana na območju Triglavskega narodnega parka (TNP), kot območju z visoko stopnjo raznolikosti turistične ponudbe in Kozjanskega regijskega parka (KRP), kot območja z ozkim spektrom turistične ponudbe. Primarni podatki so bili zbrani s poglobljenimi intervjuji z glavnimi akterji. V vsakem območju smo izvedli več intervjujev, pri čemer so v posameznih primerih intervjuvanci zastopali tudi po več deležnikov. Rezultati analize so pretežno potrdili zastavljene raziskovalne hipoteze. Ugotovljeno je bilo, da je raznolikost aktivnih udeležencev in socialnih vlog bistvenega pomena kot vir stabilnosti, prožnosti, žilavosti in celovitosti družbene komponente upravljanja z naravnimi viri; da sprememba ali propad ekološkega režima ne pomeni nujno spremembe ali propada socio-ekološkega sistema; da je adaptivni okvir vodenja kritično odvisen od sodelovanja med različnimi akterji na različnih družbenih in okoljskih ravneh; da uspešno upravljanje socio-ekonomskega področja ne pomeni nujno tudi ohranjanja stabilnega / prožnega / žilavega in celovitega socio-ekološkega sistema in da inštitucije, družbena omrežja in organizacije medsebojno sodelujejo preko večih ravni. Na drugi strani je bilo ugotovljeno, da različna raven diverzifikacije turistične ponudbe ne prispeva k višji ravni stabilnosti, prožnosti, žilavosti in celovitosti socio-ekološkega sistema.

Ključne besede: podeželski turizem, zavarovana območja, prožnost, socio-ekološki sistem, razvoj podeželja, inovacije, učenje, ranljivost

1 INTRODUCTION

This study explores two protected areas in Slovenia with a focus on rural tourism. The main purpose of the research is to analyse a rural tourism situation in two areas and to explore the influence of tourism in rural areas on resilience of the region. The analysis focused on social, economic and environmental aspect of protected areas.

The study areas were selected upon two most important criteria presented below:

- Protected area established at least 15 years ago (selected parks: Triglav National Park (TNP) and Kozjanski Regional Park (KRP) as they were the only parks that were established more than 15 years ago in Slovenia);
- Extent of tourism diversification – TNP is characterized by highly diversified forms of tourism activities and infrastructure, while in the KPR the activities offered to tourists and tourism infrastructure are not so diversified yet.

The main research objective was to understand the interrelations between the development of the tourism in investigated regions (along with its specificity) and: stability, resilience robustness and integrity of the entire region.

The following list highlights the main elements of the analysis:

1. Analysing the functioning of social-ecological systems of two chosen regions in Slovenia is the general aim of the research.
2. The analysis focused on the key dimensions for a functioning system over time, namely: integrity, robustness, stability and resilience (Stirling, 2005).
3. The four dimensions were treated as interdependent.

4. The context for undertaking the analysis consisted of: ecosystem specificity, level of biodiversity, cultural values, actors' behaviour and institutional context.
5. Functioning of social-ecological systems is also conditioned by internal and external factors having an influence on the system. These factors differ from each other not only by their character (biophysical vs. socio-economic), but also by impetuosity (shocks vs. shifts). As part of the research, in each case there were four variants of factors of change identified, which have an influence on the system.
6. Adaptation processes to the identified shocks and shifts were analyzed with particular focus on two processes: institutional change and social learning.

Initially, both selected regions were analysed separately, followed by a comparative analysis of both cases which permitted identifying the distinctive ways of adaptation to changing conditions on socio-economic and natural character.

This study has above all empirical character and it presents empirical material collected with adopted assumptions and aims of the examination. The most important elements of the used theoretical framework are introduced in the following.

Regions as complex adaptive systems – the unit of analysis will be social-ecological systems which are: **(1)** systems composed of biophysical and social components, **(2)** where individuals have self-consciously invested time and effort in some type of physical and institutional infrastructure that affects the way the system functions over time in coping with diverse external disturbance and internal problems, and **(3)** those that are embedded in a network of relationships among smaller and larger components (Janssen, Andersen, Ostrom, 2003: 7).

Development patterns of complex adaptive systems

Considering the fact that in conducted analysis the dynamics of social-ecological systems will interest us, when considering the processes of adaptation the essential dimensions of system development and their changeability should be recognized. In the process of change basic aspects of analysis will be whether (and if so - in what way) the system is able to maintain its functions. The elements of the system are not homogeneous; they have different adaptive potentials. They are conditioned with the nature of changes occurring in the system and the specificity of factors having an influence on the system. We can point to the following abilities of systems (Stirling 2005, quoted from Stagl 2006: 5-6):

Resilience is the ability of a system to maintain its functions in case of episodic exogenous shocks.

Robustness is the ability of a system to maintain its functions in case of secular external change.

Stability is the ability of a system to maintain its functions in case of endogenous disruptions.

Integrity is the ability of a system to maintain its functions in case of secular internal change.

Institutional Analysis and Development (IAD) framework

Institutions will be treated by us as the element which strengthens or impairs the adaptive potential of the system in a significant way. For the purpose of this research

a theoretical frame suggested by Elinor Ostrom and its partners (Institutional Analysis and Development) (2005) will be used in the context of analysis of the institution.

Innovation and Learning Processes

Assuming that the process of adaptation is a process whereby system element change to account for modified conditions. Effective adaptation is often only possible, when innovation and learning happen. It is the ability of the system to read signals correctly and to adjust adaptive reactions accordingly. An effective process of adaptation is a necessary condition for functional innovation to happen, whereas the appearance of the appropriate innovation is the symptom of the appropriate course of the system learning.

Referring the learning process (but also phenomenon of the innovation) to the system it is however necessary to distinguish three levels of analysis in relation to which learning/ innovation will be analysed:

- individual learning / innovation,
- organisational learning / innovation,
- regional learning / innovation.

2 RESEARCH QUESTIONS AND RESEARCH HYPOTHESES

Research questions:

1. How has the tourism sector developed over the last 15 years in each region? How can the 'action situations' of tourism activities in rural areas be described? Does the type of tourism activities influence whether stability, resilience, robustness and integrity are promoted or reduced by tourism over time? How types of tourism activities are best characterised?
2. What were major disturbances in the selected areas in the last 15 years? How can they be classified into external / internal perturbations as well as shocks (single event) / shifts (ongoing change)? What were the main outcomes of specific disturbances in each of the in the study regions?
3. Which rural actors are capable of adapting to shocks or perturbations? How did they react to each of the specific disturbances?
4. Which rural actors are capable of shaping the adaptation processes after shocks or perturbations? Are they equipped with the skills and means that they need for this task?
5. Are there some institutions in Slovenia (measures or funds), which aim to buffer shocks or perturbations?

Research hypotheses

1. More diverse tourism leads to higher stability, resilience, robustness and integrity of social-ecological systems.
2. An ecological regime shift or collapse does not necessarily result in a regime-shift or collapse of the social-ecological system.
3. Good governance of the socio-economic domain does not necessarily imply maintaining a stable / resilient / robust / integer social-ecological system.
4. A diversity of actors and social roles are essential as sources of stability, resilience, robustness and integrity in the social dimension of natural resource management.

5. The main social sources of resilience are institutional redundancy, flexible social networks, social memory and organisations that bridge levels in systems of multi-level governance.
6. Institutions, social networks and organisation interact across scales.
7. An adaptive governance framework relies critically on the collaboration of a diverse set of stakeholders operating at different social and ecological scales.

3 METHODS AND DATA

For conducting the research in question we used a case study approach as a particular method of qualitative research. Here qualitative methods are justified because they are more suitable to capture the complexity of social-ecological systems and to identify (not to omit) non-linearity of processes (Berkes, Colding, Folke, 2003: 7).

The following methods were used in this research:

- **Desk research** – it was mainly used in the first stage of research when stakeholders, key informants and perturbations (shocks/shifts) were identified and general data about TNP and KRP were collected. Desk research was mainly devoted to legal documents, official statistics, reports, articles and publications about mentioned regions.
- **In-depth interviews** – most of the data for this study were collected by using interviews. Reason for using this technique is that its semi-structured character allows respondent to partially modify structure of interview and to touch on subjects which were not included initially.
- **Observation** – in fieldwork research also observations were important, such as conditions in study area, relations between different stakeholders, opinions one about another etc.

Primary data were collected in June and July 2006. Because of the limited time and resources we asked representatives of the local authority who know the situation and stakeholders involved in rural tourism in protected areas well to prepare a list of most important actors for interviews – for both cases TNP and KRP.

In TNP interviews were conducted with the following stakeholders⁴:

- Representatives of Triglav National Park Board,
- Employees from Information Centre of TNP in Trenta,
- Owners of Tourist Farms in Trenta,
- Representatives of Tourist Association of Trenta, Log pod Mangartom and Kobarid,
- Representatives of Local Community Log pod Mangartom,
- Owners of Guest Houses in Log pod Mangartom,
- Majors of Municipalities of Bovec and Tolmin,
- Employees of Local Tourist Organizations and Tourist Information Centres in Posočje,
- Representative of Kobarid Museum of First World War,
- Member of the State Parliament from Posočje,
- Employee of Angling Club of Tolmin.

In KRP interviewees were the following:

- Representatives of Kozjanski Regional Park Board,
- Majors of the Municipality of Kozje, Bistrica ob Sotli and Podčetrtek,
- Representatives of Local Community of Kozje and Bizeljsko and at the same time owners of Tourist Farms,
- Representatives of Tourist Farms in the area of KRP,
- Representatives of Tourist Associations working in KRP,

⁴ As TNP area is big, we concentrated our interviews only in Posočje region – mostly villages Trenta and Log pod Mangartom. The reason is that we used examples of shocks and shifts from there.

- Parish priest of the Catholic Church (cooperate also in pilgrimage tourism on Svete Gore),
- Representative of Marketing Service of Spa Olimia in Podčetrtek,
- Employee of Tourist Information Centre in Podčetrtek,
- Owners of Guest Houses in KRP,
- Representative of Hunter's Family of Kozje.

Detailed data about interviewed persons are because of private data confidence available only from the research institution (Biotechnical Faculty).

Sources of secondary data collected were: legal documents, official statistics, reports, articles, scientific and other publications and other documents, internet resources – reports, self-government bodies and organisations websites. A crucial source of information were the following websites:

- home page of TNP (<http://www.tnp.si>)
- home page of KRP (<http://www.kozjanski-park.si> and <http://www.gov.si/kp/>)
- home page of Ministry of Environment and Spatial Planning (<http://www.gov.si/mop/>)

3.1 Description of perturbations within the social-ecological system

Table 1: Overview of perturbations

Perturbations			TNP - Trenta Diverse tourism	Kozjanski RP Not diverse tourism
Shock arising from socio-economic sphere	External	Shock	Independence of Slovenia and accession to EU; Closing of mine Rabelj	Independence of Slovenia and new border with Croatia; Accession to EU
		Shift	Political system change	Political system change
	Internal	Shock	Initiatives for a new Law on TNP and initiatives for changing the borders of park	
		Shift	Emigrations from area	Emigrations from area
Shock arising from geophysical sphere	External	Shock	Earthquake in Posočje in 1998	Earthquake in Kozjansko in 1974
		Shift	Growth of game (reed and roe deer, bear, wolf, and lynx)	
	Internal	Shock	Landslide in Log pod Mangartom	Drought in 2003
		Shift	Farming land overgrowing, Decreasing of Soča trout population	Farming land overgrowing

Short description of perturbations:

- Independence of Slovenia and new border with Croatia – Slovenia became independent in 1991 and a consequence was also a new border with Croatia which influenced on situation in Kozjansko region also. People from area before sold their products mostly in Zagreb market, people from Zagreb were also often visitors of the area. Border changed the situation because duties and other border limitations made obstacles for free trade (except for the illegal one). Independence of Slovenia at the same time brought more power to local communities and their development (local self-government reform).
- Slovenian accession to European Union – after some years of adaptation Slovenia joined EU in 2004. It was necessary to adopt to EU legislation but on the other hand there were new possibilities for projects and finances from EU funds.

- Closing of mine Rabelj - happened in 1991 and had influence on life in Log pod Mangartom and surrounding area, more people lost their working places, some of them after also emigrate from the area.
- Political system change – Slovenia started with political system reforms in 1989 when more parties system changed old one party socialist system. Consequences were ex-Yugoslavian market loss, market economy instead of planning economy, problems in adoption to the new system and market economy in factories etc.
- Initiatives for a new Law on TNP – the Law on TNP is still from 1981, so still from ex system and many times also in some contrary with new objectives and systems. Even more initiatives, also experiments of new law are present, so also Slovenian government had to establish a group to prepare a new Law on TNP.
- Emigrations from area – in both study cases emigrations are present and they are mostly result of lack of working possibilities and less developed infrastructure (communal, social...).
- Earthquake in Posočje in 1998 caused a lot of damage in Posočje area. Renewal was supported, managed and organized also from national level.
- Earthquake in Kozjansko in 1974 was a natural catastrophe for less developed Kozjansko region. On the other hand it contributed a lot to a new development initiative of the region (infrastructure, new buildings, tourism development...).
- Growth of game – population of wild animals sometimes increase too much and exceed the borders, as a consequence there can be a damage in agriculture, destroyed natural balance, also big fear of local population (in case of bears for example).
- Landslide in Log pod Mangartom happened in November 2000. Approximately 1,000,000 m³ of material was displaced from an altitude of 1400 to 1600 m and mainly deposited at an altitude of 630 m. The consequences of the landslide, which, by its size (materials from an area of over 25 hectares were displaced and deposited over more than 15 hectares) is one of the largest in Slovene history, are catastrophic. 7 persons lost their lives, 6 residential and farm buildings were destroyed, and another 23 buildings in the village of Gornji Log were more or less damaged. With 2 bridges ruined, the road connection between Bovec and Predel, which is of vital importance to the area, was cut off. The road to Mangart was partially or fully buried beneath earth or destroyed, and considerable damage was also caused to power supply facilities. Direct damages are estimated at almost 2 billion SIT.
- Farming land overgrowing is typical for all Slovene remote and less favoured areas, we can find some case in flat land area also. Reasons are different: not possible use of machinery on slopes, aging farm population, stopped farming activities of land owners etc.
- Decreasing of Soča trout population was mostly a result of human intervention in natural system. Some trout breeders brought a not autochthonous brook trout which was much more aggressive as autochthonous one.
- Drought in 2003 – whole Slovenia territory was affected with longer dry which had influence mostly in agriculture, water supply and fire endanger.

4 RELATIONSHIPS BETWEEN RURAL TOURISM AND THE VULNERABILITY OF REGIONS

In the following subsections a reply to questions whether regions of two explored national parks differ in their adaptation potential given in relation to individual features of social-ecological system as: resilience, robustness, integrity and stability, is given on the basis of interviewees replies.

4.1 Influence on system stability

The stability of the system can be assessed by analyzing the reaction on the closure of the Rabelj mine in TNP and establishment of the border to Croatia for KRP.

In connection to the establishment of border and loss of markets, we can say that this event was really a shock for the region and it took some time to adopt to it, and this adoption took a kind of evolutive path from “what to do now” over smuggling to redirecting to new more distant markets and change of structure of the products (from vegetable to more fruits and wine and rural tourism). The same kind of adoption happened in touristic industry (local SPA), so that on the long the area don't feel that the border presents a development problem.

In case of TNP and Log pod Mangartom the closure of the mine had both: positive and negative effects. The positive is that water pollution from the mine stopped, but on the other hand a lot of local inhabitants lost their jobs with little possibility to find a new one. The village itself did never really try to compensate this loss with some other activity, but over time the activities of surrounding area and the park (development of tourism in Soča valley and development programs such as organic farming in National park) have produced new opportunities. But still a number of people left the village.

4.2 Influence on system resilience

Regarding the change of political system and accession to the EU it is hard to tell which area adapted better. At the beginning the area of TNP had better chances to use the instruments available because of the accession process (EU financed projects) and also the private initiative could start to develop sooner (experience from neighbouring countries and national parks as well as more financial sources from people who were working over border), but also the KRP area did use the available possibilities well and is increasing its use nowadays (structural funds, international cooperation on projects etc.).

4.3 Influence on system robustness

Regarding the system robustness we could somehow conclude that both areas tried to use the available opportunities (tourism development, introducing new products, engaging in rural development programs and projects etc.), where the effect of this seems to be better in the KRP than in the TNP. The reason for this might lie in the fact, that both protected areas are of different size. The KRP is smaller and more homogeneous, so also the coordination among actions and projects is easier. On the other side the TNP is generally divided on three almost not connected valleys (because of natural barriers-mountains) with different needs and visions of future development.

4.4 Influence on system integrity

With reference to the integrity of the regions if we try to assess it over the problem of out migration then we cannot really notice the difference, because in both regions the

problem persist and for the time being also the development of tourism cannot reduce it.

But on the other hand in both regions was also mentioned, that the development of tourism is inducing the inflow of people, especially those who want to build there their second houses, what brings with a new problem: so called “black building” - building of second houses without spatial plan and permissions. Trenta valley has more development problems also because of “black buildings” and different interests of local people and weekenders.

5 CONCLUSIONS

Protected areas become one of the most important society valuableness and already now present an important part of tourist infrastructure (information centres, trails, accomodations...) and tourist offer. Data shows (Hladnik, 2005) that more as 30% of foreign tourists come in Slovenia mostly because of unspoiled nature and natural valuablenesses and also that 30% of Slovene people spend their free time in nature. In the future protected areas will be one of the top themas in tourism development. They mean »above standard offer« with peace, unspoiled nature, with nature connected activities. Slovenia has great possibilities for sustainable tourism in protected areas because of big varieties, the question is just what kind of tourism and activities are appropriate for protected areas and in what extent. It depend on each protected areas characteristics also.

The conclusions are presented in two dimensions. In the first one, we will discuss the results in relation to three issues: innovation, learning and reducing vulnerability. In the second one, we will briefly comment on answers for research hypotheses on the base of results of the research.

5.1 Innovation within the adaptive process

The appearance of the appropriate innovation is the symptom of the effective adaptation to the changing context and shocks or shifts. In the case of both investigated regions it is possible to identify the occurance of innovations.

TNP has developed its own scientific research institute which work as independant institut in frame of TNP Public Institution from 1998. The main task of institute is to collect and to arrange the results of scientific researches in park from different areas of work and to stimulate and directing the researches of research institutions and individuals and to research natural and cultural heritage. Data are useful than for natural and cultural heritage valuating. Quality informations are key importance for protection.

TNP opened also some Information Centres of TNP: one in Trenta (it helped a lot to Trenta development, mean also working places and it is a motor of development) and one in »Pocarjeva domačija« in Radovna. TNP was selected also as a partner in pilot project »Young Ranger« - innovative way to present ranger's work to pupils and to stimulate them for nature protection and to share the awareness in local communities.

In TNP they are developing also a model of eco tourism as a way of sustainable tourism appropriate for protected areas.

In Trenta TNP Information Centre together with Tourist Association Trenta prepared also innovative tourist programme "Four easy seasons" with protected trade mark. Programme foresees development of additional offer in spring and autumn, prolong of summer season and partly also of winter season. They are preparing also a new valley web portal which will present a valley completely and make possible also a central reservations system for all offerers in valley.

The innovations for Slovene management of protected areas are also the establishment of park's administration own research unit, as well as volunteer ranger service in the park, project young ranger and information centers spread across the park, which represent also kind of local development cores. Innovation resulting from natural disaster in Log pod Mangartom is also the implementation of alarming system, which is the first of its kind in Slovenia.

KRP also developed some really innovative products and projects based on natural and traditional cultural heritage of Kozjansko (based on endogenous potential). They started to evident flora and fauna and found on their area beside all 37 kind of natural orchids grow (of 60 known in Slovenia) and around 120 different birds. They also detailed evidenced rural architecture (more than 3000 units, 800 of them can have a status of cultural monument). Very innovative is also project »Kozjansko apple« and all products developed from them and trade mark SOŽITJE (symbiosis) for these products. It is not every kind of apple but old autochthonous cultivars, some of them are known only in their area (for example »sevniška voščenska«), the other are old autochthonous Slovene cultivars. Such apple cultivars are produced in traditional high trunk meadow orchards which are very important also as habitats for different kind of birds and other animals. Meadows with apple trees are late cut (not more than twice in year), so they allow also orchids to blow. They are important also as an element in traditional cultural landscape of Kozjansko so they renewed them and also planted new ones. But it is important also how to care for such orchards so they organized also education programme how to cut the trees, how to make different products from them (brandy, vinegar, juice..). As a result of education a special group of »tree cutters« were qualified and they went around and cut the trees and learn people in area how to do that. Trade mark for products from apples was protected in Patent Office. They found also a special way of bottle filling – it enables to store a juice for two years without conservans. At the end »apple project« finished also with now traditional international »Apple festival« (professional, seminar, cultural and social event). Renewal of high trunk meadow orchards became also an international (INTERREG) project. In Kozjansko also joinery was traditional and "apple project" brought new possibility for revival also-to make some products from very colourful apple or nut tree wood. KRP together with partners from Finland, Germany and Austria established also partnership network EUREX 21 for information and experiences exchange, presenting their products, projects and culture. Innovative is also breeding of capons under meadow orchards as revival of an old Middle Ages Characteristics. Within the cultural programmes of the Kozjanski Park also Music Summer at the Podsreda Castle is innovative. Also different walking trails on area are innovative and also some new initiative for eco village. In one abandoned hamlet

with four homes they want to develop ecological village with apartments and with parallel tourist and educational offer.

5.2 Learning within the adaptive process

Summing up the issue of learning it is worthwhile mentioning that the effective process of learning must co-occur with the process involving all actors into the process of deciding and managing protected area and its surroundings (Kofinas 2003: 7). In both case such action is being taken. Main initiator of these actions in both areas is the park administration, where the TNP is much more active due to its bigger size, higher financial support from national budget and longer existence. The active learning is organized in forms of different workshops, seminars, participation in national and international projects and transfers of good practices for different kind of people. Both parks administrations are also putting a great effort on cooperation with children, so in both areas they are trying to involve actively local schools into the park activities.

As the both parks are in the moment in the process of preparing their management plan they put also a great effort to involve local actors into this process to learn what the local and interested public expect from the park, what are their needs and problems etc.

5.3 Reducing vulnerability by promoting rural tourism

All available data (primary and secondary) and also interviews with people from protected areas shows that rural tourism is seen as a very important, probably also the most important, factor for economy in area and also as a solution for lost working places in both study areas. But not mass tourism and not every kind of tourism. Both areas promote sustainable way of tourism in connection with nature protection and local area characteristics. Endogenous potentials (natural and human) of each area are most important. Tourism in park increasing and it means also important contribution in economy of individual area.

In TNP they have vision about ecotourism as an appropriate way of tourism for protected areas. Ecotourism is one way of sustainable tourism; it is an instrument for natural protection and at the same time assures sustainable economic benefits for local people (Šolar, 2005). Ecotourism can be understood as environmental, sociological and economic category. As economic category it can crucial contribute to sustainable rural development and it is at the same time a motor of development. As sociological category it can contribute to higher awareness of public about importance of nature protection, at the same time visitors have impression that with their appropriate treatment contribute to protection and maintaining. As economic category ecotourism assure promotion and marketing of products from protected areas like nature, cultural heritage, clean water, fresh air, local autentical products (also from ecological farming). But all kind of tourism activities and their development should be adjusted with local population in protected areas.

Tourism in protected areas is mostly in tight connection with agriculture also. In TNP and KRP such connections are very important and they also build their offer on them.

Agriculture maintains cultural landscape and increase attractivity of the park and products of farming are important tourist product also (Kozjansko apple case or wool products from Trenta). Tourism can also offer local produced food, prepared by local recipes for example. Extensive farming methods increase biodiversity also. In KRP Park Board want to increase number of ecological farms and to create a network between ecological farms, tourism in park and spas in surroundings.

5.4 Research hypotheses

H-1: More diverse tourism leads to higher stability, resilience, robustness and integrity of social-ecological systems.

In the case of two analysed protected areas, the region TNP has more diversified tourist offer but in the analysis we could not find a firm evidence which indicates higher stability, resilience, robustness and integrity of this area. In some cases the less divers area (KRP) proved to be better off (i.e. stability of regions).

H-2: A diversity of actors and social roles are essential as sources of stability, resilience, robustness and integrity in the social dimension of natural resource management.

On the example of two analysed cases it is possible to notice, that some actors are simultaneously performing different roles (i.e. the same person is local representative, chair person of local tourist board and owner of biggest tourist farm in the area) what leads to the better adaptation to shocks and shifts. Such a situation is taking place in both cases.

This facilitates to see at a lot of issue from different perspectives, to understand different arguments; it leads the better adaptation, higher stability, resilience, robustness and integrity.

Involving representatives of different subjects into the dialogue is also leads higher stability, resilience, robustness and integrity, what proved to be correct very evidently in the case of KRP, where with the change in the management of the park, which involved into its work more local stakeholders, increased the positive acceptability of the park by the local inhabitants.

H-3: An ecological regime shift or collapse does not necessarily result in a regime-shift or collapse of the social-ecological system.

The good example to evidence this hypothesis is KRP case with draught.

The drought had important influence in the year 2003, but rather short-lived and not causing permanent damage. Ecosystem possesses possibilities of curbing the negative influence of some process or factor, it is not a necessary interference of the man.

It is possible to say for investigated region that the draught was rather "fact media" which had no notable consequences neither on behaviour of local population (no one decided for installing irrigation system afterwards) nor on the visitors.

H-4: An adaptive governance framework relies critically on the collaboration of a diverse set of stakeholders operating at different social and ecological scales.

In both cases - TNP and KRP - research demonstrated the general adaptation connected with the free market economy. In the KRP case the adaptation to the conditions of the free market economy is more obviously connected with the development of tourism as in the case of TNP, which was already before the shift

touristically developed. Now tourist businesses are developing at all communities. It is an adaptation on the regional level in frames which is the element of cooperation connected with the fact that generally the tourism is seen as one of the development opportunities for the whole country and not only for the investigated regions.

H-5: Good governance of the socio-economic domain does not necessarily imply maintaining a stable / resilient / robust / integer social-ecological system.

1) KRP – An excellent example of cooperation of diverse groups of actors is a situation after appointing the new director of the park. After the new director involved the local stakeholders into the park management, all the interviewed actors evaluated a park as much more acceptable for the local population.

The benefits from cooperation get not only to actors but also national park, which can more effectively realize one's objectives. In the KRP case can see very well a participatory model of park management. The new director which at the moment in the verbal phase is declaring the will of the cooperation is getting considerably bigger capital of the confidence than its predecessor which was perceived as the person throwing such a model of managing with park.

2) TNP - in this case in until now cooperated three categories of actors: the park administration, representatives of the self-government and the government as the founder of the park. The relative high interest of government and park administration in the nature protection and the lack of the consistent politics of all communities in the scope of the development caused that mostly local population saw park as limitation for the development of tourism on the national park area. At present the change can be noticed in the process of preparing a new law, where the government has involved a much wider spectres of stakeholders in its preparation.

In the context of the multilevel cooperation of diverse categories of actors we need to pay attention for the special NGOs role. In the process of developing a new law they became very active and they are becoming the actor taking aiming action to make policies of self-government bodies and the management of the park more cohesive. In other words, they are becoming the crucial subject holding the model of multilevel cooperation in this way.

H-6: Institutions, social networks and organisation interact across scales.

We are analysing three levels: individual, group/organisational, and regional. The shock/shift which feld the most reactions in both parks is earthquake.

This shock has in both areas mobilized the higher number of both formal and informal institutions on local and national level. The local level institutions were mostly active at very beginning at helping people to rescue their lives and property, and they started to reduce their activities as the eminent treath to this reduced or finished. On the other hand the national level institutions became more active in next phase in reconstructing the caused damages.

At the beginning the activity of all this institutions was very high and different cooperations between them were established, but when the time passing these interactions started to weaken and with the time even broke or were re-established on the lower level as before the shock. In illustration to this can be a comment from one inhabitant of Log, who said that just after the quake and landslide everybody was connected to other, but after rebuilding the village, when bigger distances between houses were made, the people even became less linked together as before the disaster.

H-7: The main social sources of resilience are institutional redundancy, flexible social networks, social memory and organisations that bridge levels in systems of multi-level governance.

In the TNP regions the social memory and social networks could be recognised as the important factor enhancing the adaptive potential of governance, as the national park has a long lasting tradition (over 80 years) and because of closed alpine communities also process of social memory transmission is still present.

In the case of KRP this is not so evident; as the region is more open and less developed so the out migration was much higher.

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Grafični prikaz besedilnih pomenov v znanstvenem članku o varstvu zelja (*Brassica oleracea* L. var. *capitata*)

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IZVLEČEK

Glavni namen pričujočega prispevka je osvetliti vprašanje besedilnih vzorcev v znanstvenem članku s področja varstva zelja (*Brassica oleracea* L. var. *capitata*). Teoretično izhodišče za analizo je sporočilna semantika, ki se naslanja na sistemsko-funkcijski model jezika. V prispevku so uporabljene metode besedilne analize v povezavi z orodji korpusnega jezikoslovja. Pri beleženju jezikoslovnih značilnosti, nanizanih v linearnem poteku besedila, ki bi jim težko sledili z ročnim označevanjem, nam je v pomoč oblikoslovni označevalnik za slovenščino, izdelan na Inštitutu za slovenski jezik ZRC SAZU, pri izpisovanju jezikoslovnih značilnosti besedil pa je uporabljena grafična predstavitev. Graf je nekakšen slikovni model in je tako izhodišče za opis besedilne zgradbe znanstvenega članka.

Ključne besede: zelje, *Brassica oleracea* L. var. *capitata*, varstvo rastlin, znanstveni članek, besedilni vzorci, oblikoslovni označevalnik za slovenščino

ABSTRACT

MAP OF TEXTUAL PATTERNS IN RESEARCH ARTICLE OF PROTECTION OF CABBAGE (*Brassica oleracea* L. var. *capitata*)

This paper aims to explore textual patterns of research article of protection of cabbage (*Brassica oleracea* L. var. *capitata*). The theoretical construct for the analysis is provided by concept of message semantics as defined in Systemic Functional linguistics. In the study, the specificity of discourse analysis is combined with tools of corpus analysis. The linguistic features of texts which would be difficult to keep track of by manual tagging are recorded by the automated part-of-speech tagger for Slovenian developed at the Institute of Slovenian language at ZRC SAZU and the graphic display is used for the presentation of data. The graph functions as a sort of map and as such becomes the starting point for the description of the discourse structure of research article.

Key words: cabbage, *Brassica oleracea* L. var. *capitata*, crop protection, research article, textual patterns, part-of speech tagger for Slovenian language

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UVOD

Da besedilo ni samo linearna združba stavkov, ampak hierarhično urejena struktura, je teoretično izhodišče vseh novejših smeri jezikoslovja, sociologije in psihologije, ki se ukvarjajo s preučevanjem besedila. Zgodovinski pregledi nam kažejo, da ima preučevanje zgradbe besedil v literarni vedi dolgo tradicijo in je spodbudilo tudi raziskovanje strukturne zgradbe besedil v jezikoslovju. S problematiko strukturne zgradbe besedil se na evropskih tleh ukvarjajo vse od antike. Tako je že Aristotel v *Poetiki* (ok. 330 pr. Kr.) obravnaval zgradbo tragedije in epa (Aristotel, 1982). Strukturni zgradbi se posvečata predvsem dve jezikoslovni šoli: sistemsko-funkcijska in pragmatična.

Teoretično izhodišče prispevka je model za besedilno analizo, kakršnega je izoblikovala Hasanova (Hasan, 1984). Zaradi tesne navezanosti njene teorije na Hallidayevo teorijo jezika smo najprej zarisali širši tloris Hallidayeve misli o jeziku. Pri obravnavi vprašanja pomena v Hallidayevi teoriji jezika je treba postaviti v izhodišče dejstvo, da gre za sistemsko-funkcijsko teorijo jezika, ki poudarja, da jezik ni samo abstrakten sistem znakov, ampak da je njegova osnovna funkcija sporazumevalna, zato je tudi razumevanje pomena zelo široko. Po Hallidayu je vsaka jezikovna dejavnost vpeta v družbeno-kulturni kontekst. Pri sporazumevanju govorec iz mreže jezikovnih pomenov izbere samo nekatere pomene. Proces in rezultat pomenskega izbora v določenem kontekstu je besedilo. In čeprav se nam govorjena in pisna besedila na prvi pogled kažejo, kakor da so iz besed in stavkov, so v resnici zgrajena iz ubesedenih pomenov (Halliday, 1989). V sistemsko-funkcijski teoriji jezika je besedilo pomenska enota.

Opis pomenskega ustroja besedila je zahtevna naloga, saj je besedilna semantika novo področje jezikoslovja, ki je v primerjavi z besedno in stavčno semantiko dokaj neraziskano. Glavni razlog za težko razumevanje semantičnega ustroja besedila je neizdelan metajezik za opis te ravnine (Birch in O'Toole, 1988).

METODE DELA

Metajezik za opis besedilnega pomena

Podlaga za analizo semantičnega ustroja znanstvenega članka je bila sporočilna semantika in njeni semantični pojmi: sporočilo, entiteta in dogodek (Hasan, 1991). V sistemskem jezikoslovju je temelj za oblikovanje pomenskih mrež opis oblikoskladenjskih vzorcev, saj je med pomensko in slovnično ravnino dialektično razmerje. Pomeni so dosegljivi samo kot ubesedeni pomeni na slovnični ravnini (Halliday, 1973). Človekove predstave postanejo v besedilu jezikovni izrazi ali modeli: entiteta, dogodek in okoliščine, ki so v stavku izraženi kot samostalniška zveza, glagolska zveza in prislovne ali predložne zveze. Zato je prvi korak k analizi besedil opis oblikoskladenjskih značilnosti osebkovih entitet in povedkovih dogodkov. Ker postopek ročnega vnašanja oblikoskladenjskih značilnosti ni primeren za analizo daljšega besedila, smo se odločili za oblikoskladenjsko označevanje, ki je predstavljeno v naslednjem razdelku.

Oblikoslovno označevanje

Za bolj detajlno jezikoslovno analizo besedil moramo računalniško besedilo označiti dodatno. Tako kot imamo v jezikoslovju različne ravnine, na katerih preučujemo jezikovne enote, je tudi v korpusnem jezikoslovju mogoče označevanje na različnih ravneh: glasoslovno označevanje (angl. 'phonological annotation'), oblikoslovno označevanje (angl. 'grammatical

annotation' ali 'part-of-speech tagging'), skladenjsko označevanje (angl. 'syntactical annotation'), semantično označevanje (angl. 'semantic annotation') in pragmatično označevanje (angl. 'pragmatic annotation') (O'Donnell, 1999).

Najbolj razširjena oblika jezikoslovnega označevanja je oblikoslovno označevanje. Oblikoslovno označeni korpusi se ponavadi uporabljajo za leksikografske, oblikoslovne in skladenjske raziskave posameznih jezikovnih značilnosti. Da je oblikoslovno označeni korpus mogoče uporabiti tudi za besediloslovne raziskave, je pokazal Biber (1998). Kako se glagolske oblike nizajo v besedilu, je prikazal z diagramom, iz katerega je jasno vidno, da meje med deli besedila niso ostro začrtane, ampak zajemajo konec ene enote in začetek nove enote. Za oblikoslovno označevanje potrebujemo posebno programsko opremo in nabor oznak. Označevalnik za slovenščino je začel nastajati leta 1996 na Inštitutu za slovenski jezik ZRC SAZU in je bil prvič predstavljen v Slavistični reviji (Jakopin in Bizjak, 1997).

Izhodišče za našo analizo znanstvenega članka s področja varstva zelja je torej oblikoslovno označeno besedilo. Na sliki je 1 ponazorjen odlomek oblikoslovno označenega besedila na računalniškem zaslonu.

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§V_ letu 2002 smo_ preučevali vpliv števila škropljenj z_ deltametrimom na zmanjšanje
§E5 Sse5 Š GPap GLmp Sme4 Sse2 Ssp2 E6 Sme6 E5 Sse4
škodljivosti tobakovega resarja na zgodnjem zelju.
Sže2 Sme1 E5 Pse5 Sse5
§V_ bločnem poskusu smo_ na zunanjih listih glave občutljivega cv. Pare1 ugotavljali odstotek
§E5 Pme5 Sme5 GPap E5 Pmp5 Smp5 Sže2 Pme2 K IRme1 GLmp Sme4
poškodovanosti listne površine.
Sže2 Pže2 Sže2
§Ob upoštevanju nižjega gospodarskega praga škodljivosti (do 1% poškodovane listne površine) med
§E5 Sse5 Pme2j Pme2 Sme2 Sže2 E2 PNže2 Pže2 Sže2 E6
obravnavanja, v_ katerih smo_ rastline enkrat in_ dvakrat poškropili z_ insekticidom, ne
Ssd6 E5 ZRsd5 GPap Sžp4 A Vpr A GLmp E6 Sme6 ČZ
ugotavljamo razlik.
Gap Sžp2
§Omenjeni prag tam ni_ bil_ presežen šele pri osmem zunanjem listu v_ glavi, kar je_
§PNme1i Sme1 ZK GZPce GLBme GNme1 Č E5 ŠVme5 Pme5 Sme5 E5 Sže5 ZR GPce
veljalo tudi za neškropljene rastline, pri katerih smo_ na zunanjih listih ugotovili največji
GLse Č E4 Pžp4 Sžp4 E5 ZRžp5 GPap E5 Pmp5 Smp5 GLmp Pme4JJ
povprečni indeks poškodb.
Pme4i Sme4 Sžp2

```

Slika 1: Odlomek oblikoslovno označenega besedila

Figure 1: Section of grammatically tagged text

V oblikoslovno označenem besedilu ima vsaka beseda pripisano pravilno, od sobesedila odvisno oblikoskladenjsko oznako. Oblikoslovno oznako sestavljajo: kratica za besedno vrsto, ki je pisana z velikimi tiskanimi črkami in vedno na prvem mestu, sledijo kratice, pisane z velikimi tiskanimi črkami, ki označujejo besednovrstne skupine, in kratice za oblikoslovne značilnosti, ki so pisane z malimi tiskanimi črkami ali števki. Nabor oznak sta podrobneje opisala Jakopin in Bizjak (1997).

Glavni namen oblikoslovnega označevanja je bil pojasniti teoretične pojme, ki so vključeni v pojmovni sestav sporočilne semantike in oblikoslovnega označevanja.

Analiza znanstvenega članka

Zapleteno vprašanje besedilnega pomena, to je prepoznavanje semantične kontinuitete in premikov ter s tem povezano razmejevanje strukturnih enot, smo ponazorili z besedilno analizo znanstvenega članka: Vpliv števila škropljenj z insekticidom na zmanjšanje škodljivosti tobakovega resarja (*Thrips tabaci* Lindeman, Thysanoptera, Thripidae) na zgodnjem zelju (Trdan in Žnidarčič, 2004).

Določanje osebkovih entitet in povedkovih dogodkov je bilo v prvem koraku opisovanje oblikoskladenjskih uresničitev osebkov in povedkov v stavčnih enotah. Postopek ročnega vnašanja oblikoskladenjskih značilnosti je zelo dolgotrajen že za eno besedilo. Oblikoslovno označeno besedilo nam je omogočilo samodejno beleženje jezikoslovnih značilnosti,

nanizanih v linearnem poteku besedila. Tako v nadaljevanju predstavljamo računalniški postopek, s katerim smo iz oblikoslovno označenega korpusa dobili izpisek zaporedja glagolskih in samostalniških oblik, kakor si sledijo v linearnem poteku besedila, in predstavili postopek pretvorbe niza oblikoskladenjskih oznak v grafični zapis.

Grafični prikaz osebkovih entitet in povedkovih dogodkov

Z oblikoslovnim označevalnikom za slovenščino, vgrajenim v urejevalnik EVA, smo označili izbrani znanstveni članek, kar omogoča samodejno zapisovanje jezikoslovnih značilnosti, nanizanih v linearnem poteku besedila. Glagolske oblikoskladenjske oznake smo glede na kategorijo osebe, števila in časa pretvorili v številčne oznake in v programu MsExcel oblikovali graf.

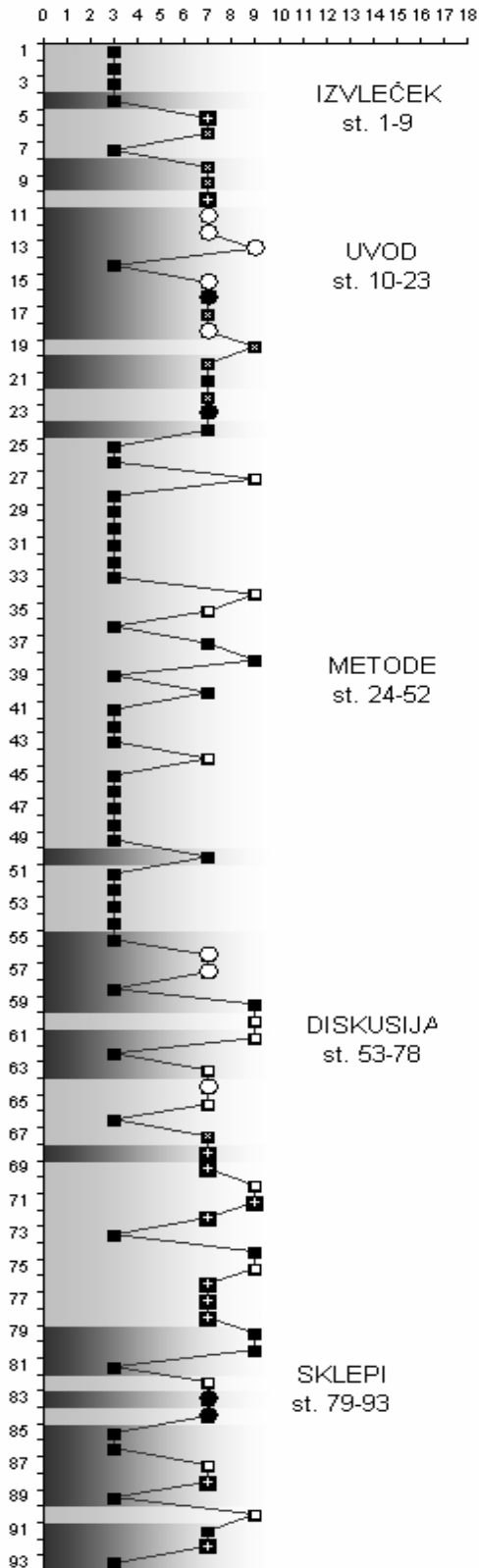
REZULTATI

Graf, ki je bil izdelan na osnovi podatkov o osebkovih entitetah in povedkovih dogodkih v članku, je prikazan na sliki 2 in je opremljen s pojasnili za branje.

Enote od 1 do 9 na vodoravni osi označujejo kategorijo osebe in števila. Enote na navpični osi pomenijo zaporedno številko stavka v besedilu. Osebkove entitete so predstavljene kot točke in povedkovi dogodki kot polja.

V prvem stavku, ki je na navpični osi označen z zaporedno številko 3, je entiteta v smeri vodoravne osi oddaljena za tri enote, kar pomeni, da je nanosnik govorec in raziskovalci (1. oseba množine), barvno polje pa je svetlo, kar nam sporoča, da je dogodek prvega stavka usmerjen v sedanost. Tudi nanosniki drugega, tretjega in četrtega stavka so raziskovalci, v četrtem stavku pa je dogodek usmerjen v preteklost, saj je barvno polje temno. Razpon od 1. do 6. enote na vodoravni osi je razpon netretje glagolske osebe in na tem območju grafa se gibljejo črni kvadrati, ki sestavljajo pomensko skupino, imenovano raziskovalci. S črnim kvadratom so označene tudi tiste entitete, ki se gibljejo v razponu tretje glagolske osebe, a se nanašajo na raziskovanje (na primer 21. stavek). Razpon tretje glagolske osebe je od 7. do 9. enote na vodoravni osi in na tem območju so razvrščene še naslednje pojmovne entitete: zelje (beli kvadrat), tobakov resar (beli krog), deltametrin (črni krog) in pojem škodljivost (plus).

Da bi pojasnili, kako so v grafu upodobljene osebkove entitete z barvnimi točkami in povedkovi dogodki z barvnimi polji in kako je zabeleženo njihovo gibanje, ali drugače povedano, kako je treba brati graf, smo v tem razdelku podrobno opisali entitete in dogodke za nekatere stavke besedila. Vendar namen grafa ni branje stavka za stavkom. V nadaljevanju smo graf uporabljali kot slikovni model, ki smo ga vzeli pod drobnogled in opazovali besedilne vzorce, ki jih ustvarja zaporedje entitet in dogodkov v tem besedilu.



LEGENDA:

vodoravna os = kategorija osebe in števila

- 1 = 1. oseba ednine
- 2 = 1. oseba dvojine
- 3 = 1. oseba množine
- 4 = 2. oseba ednine
- 5 = 2. oseba dvojine
- 6 = 2. oseba množine
- 7 = 3. oseba ednine
- 8 = 3. oseba dvojine
- 9 = 3. oseba množine

navpična os = zaporedna številka stavka

osebkovalna entiteta

- črni kvadrat = raziskovalec, poskus
- beli kvadrat = zelje
- beli krog = tobakov resar
- črni krog = deltametrin
- plus = škodljivost
- križec = drugo

čas povedkovega dogodka

- temno polje = preteklik
- svetlo polje = sedanjik

Slika 2: Graf za znanstveni članek o varstvu zelja
 Figure 2: Map of research article of protection of cabbage

Opis besedilnih vzorcev

S pomočjo slikovnega modela na sliki 2 smo osvetlili besedilne vzorce, ki jih v besedilu zarisuje gibanje entitet in dogodkov. Že bežen pogled na slikovni model znanstvenega članka je dovolj, da opazimo razlikovanje med entitetami na začetku in koncu besedila ter entitetami v osrednjem delu. Kakšne so torej razlike med začetnim in osrednjim delom? Kaj pa med začetnim in končnim delom?

Poglejmo si enoto, imenovano izvleček, in opazujmo vzorce, ki jih ustvarjajo konfiguracije entitet in dogodkov. Od prvega do devetega stavka prevladuje entiteta, ki označuje raziskovalce v prvi osebi množine (črni kvadrat), dogodki pa so usmerjeni v sedanost in preteklost. Pomeni se torej gibljejo v pojmovnem polju raziskovanja.

Prehod k novi enoti, uvodu, uvaja deseti stavek. Iz grafa lahko razberemo, da v uvodu ustvarjata kontinuiteto na osi entitet raziskovanje (črni kvadrat) in uničevanje (entiteta tobakov resar, ki je predstavljena z belim krogom, in entiteta deltametrin, ki je predstavljena s črnim krogom). Na časovni osi pa prevladuje usmerjenost v preteklost. Naslednja enota se razteza od 24. do 52. stavka in se imenuje metode. Tudi metode so tako kakor izvleček raziskovalno osredinjena enota (črni kvadrat), ki uvede entiteto zelje (beli kvadrat). Lastnosti, ki gradijo pomenski premik v metodah, so vezane tudi na os dogodka, na kateri je izbrana možnost za sedanost, to je istost časa govorjenja in pomenske podstave.

Od 53. do 55. stavka imamo prehod k enoti diskusija z rezultati, za katero je značilno, da so entitete bolj razpršene. V linijo kratkih sledij, najprej tobakov resar in zelje (beli krog in beli kvadrat), nato pojmovna entiteta škodljivost (plus), se nenehno vpleta entiteta raziskovanje (črni kvadrat). Povedkovi dogodki so v prvi polovici enote usmerjeni v preteklost (temno polje), v drugi polovici v sedanost (svetlo polje). Taka razpršena linija sledij se nadaljuje tudi v sklepni enoti (stavki 79–93), vendar z osredinjenostjo na preteklost.

Z analitičnim opazovanjem besedilnih vzorcev, ki jih ustvarjajo entitete in dogodki, smo za znanstveni članek o varstvu zelja opisali pet jasno zamejenih delov besedila. Ugotovili smo, da se deli besedila, to so izvleček, uvod, metode, diskusija z rezultati in sklepi, precej razlikujejo glede na konfiguracije entitet in dogodkov. Osredotočenost na raziskovanje, sledja pojmov s področja varstva zelja ter raba preteklega in sedanjega časa so lastnosti, ki oblikujejo znanstveni članek.

Graf je torej nekakšen slikovni model, ki beleži gibanje entitet in dogodkov in je tako izhodišče za opis strukturnih vzorcev, nastajajočih s kontinuiteto, premiki in prepleti entitet in dogodkov v besedilu. Prav grafična upodobitev s svojo statičnostjo naj bi nam pomagala, da tisto, kar ostaja v ubeseditvenem procesu neopazno in prikrito, pusti sled, pride na površje ter nam da predstavo o kontinuiteti in premikih v pomenskem valovanju besedila.

SKLEPI

Z besediloslovno analizo smo ugotovili, da pozorno opazovanje nizanja osebkovih entitet in povedkovih dogodkov pripelje do strukturnega tlorisa znanstvenega članka s področja varstva zelja. Poseben pomen pri opisu pomena sta imela semantična pojma entiteta in dogodek, ki se navezujeta na sporočilno semantiko (Hasan, 1991) in s katerima je mogoče opisati strukturne enote besedila in narediti pomenske premike, ki so v jeziku zakriti in jih poslušalec/bralec zaznava le intuitivno, razvidne in dostopne jezikoslovnemu preučevanju.

Čeprav je namen prispevka grafično prikazati besedilne vzorce, se ob analizi postavljajo nova vprašanja o besedilnih vzorcih v znanstvenih člankih s področja varstva zelja. Da bi dobili gradivo za analizo, ki s svojim obsegom zagotavlja tudi ustrezno reprezentativnost vzorca za dovolj zanesljivo in veljavno posplošitev rezultatov na populacijo znanstvenih člankov s tega področja, bi morali oblikovati korpus, ki bi obsegal najmanj 50 besedil. Tako je prispevek tudi spodbuda za nadaljnje zbiranje gradiva in ugotavljanje besedilnih značilnosti znanstvenih člankov s področja vrtnarstva in varstva rastlin.

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Agrovoc descriptors: landscaping; methods; aesthetic value; ornamental value; value systems; plant anatomy; plant habit; plants; ornamental woody plants; shrubs; trees; identification

Agris category code: P01, F50, F70

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Visual characteristics as a key factor in species selection in vegetation planes design^a

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ABSTRACT

In the past, the selection of plants used to rest upon individual taste and fashion dictates of the period, rather than starting from and following directives determined by the goal of creating a certain space characteristics. In the literature the plant species are frequently treated individually and arranged according to the visual effect of each plant. In order for this research to satisfy the needs of design practice, it was necessary to ascertain the hidden designer potential of each plant, which is not identical with the pleasing effect of the plant. This is how the central problem is formulated in this research: to determine the selection criteria (of plant species), arising from the visual characteristics of the plants and, based on these criteria to determine the suitability of plants in order to create visual effects of vegetation planes. In this sense we can expose the importance of proving the connectedness between morphological properties of the plants and the characteristics of vegetation planes, which are formed by these very plants. The subject of this research is plant material, 208 shrubs and 193 trees, systematized according to their size, shape, habitus, texture, colour, as well as the seasonal appearance of individual characteristics. We established the method and criteria for the selection of morphological properties of the plants that allow us to achieve a certain visual character of the vegetational plane. The process of plants selection is shown on concrete examples, whereby certain design demands determine the choice of the adequate plants. For the final selection of plants it is necessary to add criteria arising from eco-physiological needs of the plants and from technological demands.

Key words: landscape architecture, methods, plant material, plant morphology, vegetation plane, selecting species

^a The article is a part of doctoral dissertation, supervisor Ph. D. Professor Ana Kučan

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IZVLEČEK

METODA UPORABE MORFOLOŠKIH LASTNOSTI KOT MERILA ZA IZBOR RASTLINSKIH VRST PRIMERNIH ZA IZGRADNJO VEGETACIJSKIH PLOSKEV^b

Izbor rastlinskih vrst je pogosto temeljil na okusu in modi dobe, bolj kot na nizu izhodišč, določenih s ciljem ustvarjanja določenih prostorskih značilnosti. V literaturi so rastlinske vrste pogosto obravnavane individualno in razporejene glede na vidne učinke posamezne rastline. Da bi raziskava zadostila potrebam oblikovalske prakse, je bilo potrebno ugotoviti skriti oblikovalski potencial rastline, ki pa ga ne enačimo z všečnostjo rastline. Tako je postavljen osnovni problem v raziskavi: določiti merila za izbiranje rastlinskih vrst, ki izhajajo iz vidnih lastnosti rastlin in na podlagi teh meril določiti ustreznost vrst za ustvarjanje vidnih učinkov vegetacijskih ploskev. V tem smislu lahko izpostavimo pomen dokazovanja povezanosti med morfološkimi lastnostmi rastlin in lastnostmi vegetacijskih ploskev, ki jih te rastline tvorijo. Predmet raziskave je tako rastlinsko gradivo, 208 grmovnih in 193 drevesnih vrst razporejenih po velikosti, obliki, habitusu, teksturi, barvi in času pojavljanja posameznih lastnosti. Metodološko so razvita merila za izbor morfoloških lastnosti rastlin, s katerimi lahko dosežemo določeno vidno značilnost vegetacijske ploskve. Postopek izbiranja rastlin je prikazan na konkretnih primerih, kjer se na podlagi določene oblikovalske zahteve izberejo ustrezne vrste. Za končni izbor rastlinskih vrst pa je potrebno dodati še merila, ki izhajajo iz ekofizioloških potreb rastlin in tehnoloških meril.

Ključne besede: krajinska arhitektura, metodologija, rastlinsko gradivo, morfološke lastnosti, vegetacijske ploskve, izbor rastlin

1 INTRODUCTION

The knowledge about plant material and its applicability is an important factor in achieving quality in landscape design. It manifests in the appropriate selection of plant species for individual design solutions.

In nature the distribution and expansion of plants is determined by biotic factors (soil, climate, fauna) whereas in a designed landscape humans represent the main factor. Here the plants become material, and humans make choices, arrange and remodel according to their needs and wishes.

In the past – and it is often still so nowadays - the selection of plants was wont to rest upon individual taste and fashion dictates of the period, rather than starting from and following directives determined by the objective of creating a certain space characteristics.

The primary elements or rather ‘building blocks’ of a designed landscape are called landscape elements. These can be vegetational (lawn plane, tree avenue) or non-vegetational (water and sand planes, buildings) (Ogrin, 1996). Examining the relevant literature (Zion, 1970; Robinson, 1992; UCONN Plant Data Base of trees, shrubs and vines, 2005; Wyman, 1956) it was ascertained that the research of plant suitability as regards the construction of vegetational plane phenomena had been fragmentary. Merely a few basic elements have been treated, such as hedges and ground-cover plants. The procedures of selection are non-transparent and quite inadequate for the use of serious design.

^b Prispevek je del doktorske naloge, mentorica izred. prof. dr. Ana Kučan

Therefore the main research problem can be seen as determining criteria for the selection of plant material, arising from the visual properties of the plants and, based on these criteria, subsequently determining the suitability of plants as to their capacity to create visual effects of vegetational planes. This paper focuses on vegetational planes; it is in this light that the objective is set - examining the morphological properties of plant species with the aim to establish a means of selection of plant species suitable for the construction of vegetational planes.

2 VEGETATIONAL PLANES

Vegetational planes represent one of the possible plane features (water, rock, sand, buildings, vegetation). In terms of their direction in space they can be horizontal, slanting or vertical; in terms of surface properties they can be flat or modulated (concave or convex).

The properties of the materials by means of which a plane is composed are uniformity, homogeneity, coherency (Ogrin, 1996). Significant characteristics of the plants that form horizontal planes, are low variability within one species, equalized texture of shoots, leaves, blooms, and regular growth.



Figure 1. Vertical vegetational plane (*Pterocarya fraxinifolia*, BF, Ljubljana)

The character of the plane also depends on the size of the planting area. One of the important properties we expect from a good cover plant is its habitus. However, the suitability of the habitus is not estimated by the appearance of each individual plant. It is important to see the park as a whole. Importance is given to the holistic aspect of the park. This is where the dense growth of the different plants plays an important role (the direction of the offshoot growth, the intertwining of shoots). Good cover plants should cover the ground rapidly and form a condensed vegetational cover. This requirement is best fulfilled by quick-growing species with even growth (with no intervals of quick and slow growth).

Vertical vegetational planes occur in nature at the juncture of two different ecosystems. They can be found alongside water phenomena like river banks or lake shores. Alternatively they can develop due to the influence of geomorphologic phenomena (karstic edges, earth depressions). In the cultured landscape they occur when land use is change, like at the juncture of forest (or its remnants) and farming

land, the juncture of near-water vegetation with cultured landscape and similar. In landscape design vertical vegetational planes play a significant part in the articulation of space. They define spatial boundaries and the measurement of the space, create effects of depth, form backgrounds, sceneries and similar. In order to create an effective sense of the plane, it is important to respect the homogeneity of material, especially with regard to its vertical direction (trunk, branches, shoots). This can be accomplished by means of appropriate species selection and by choosing the appropriate planting density (Dobrilovič, 2005:18).

Properties of plant species suitable for the construction of vertical vegetational planes are uniform branch and shoot growth along the whole length of the prolongation of the trunk or main shoot, and good adaptability of the plants to the change of lighting. Furthermore the shoots and branches should sprout low (above-ground), the length of side branches and shoots should be even. A long life span, equalization within the species, clearly determined periods of pheno-phases in development (flowering, growth).

3 MATERIAL AND WORK METHOD

The subject matter of this research project are plant species designed for greening of public spaces. They are chosen on the basis of assessing their hardiness, their resistance to urban climate, to diseases and pests, the cost of maintenance and their design potential.

The research encompasses autochthonous species (Kotar and Brus, 2003), suitable for the use in public parks and foreign species or sorts of trees and shrubs, which are wide-spread in our country and fulfil users demands (Šiftar, 2001).

The principal goal of the research is to define appropriate criteria for plant species selection in order to obtain the desired effect of the vegetational plane. In order to achieve this it was first necessary to establish the connection between the morphological and the visual properties of the vegetational plane (height, texture, colour and similar) formed by the plants, and to determine which morphological properties of the plants affect the desired character of the vegetational plane.

As far as the work method is concerned it is imperative to emphasize that the aspect here treated as far as the knowledge of plants is concerned, covers a fairly unknown ground and does not provide well-established methods of research. Therefore in the continuation the work process is introduced in some detail. It was necessary to systematize all plant species (the research subject matter) according to their visual properties (size, shape, habitus, texture, colour) and the properties determined by their seasonal appearance (e.g. foliage colour – autumn, colour of blossoms – spring ...). All plants were classified on the basis of data obtained by observing visual properties in situ, and subsequently comparing them with findings of other authors. This was followed by a meticulous analysis in terms of visual properties (size, shape, colour, texture, durability of foliage). Next step was the setting of criteria for the selection of morphological plants by means of which we can achieve the desired visible characteristics feature of the vegetational plane. In order to help establish the afore-mentioned connections, in the continuation two schemes are being demonstrated in which the grey colour designates those morphological plant properties by which the character of horizontal (Figure 2) and vertical (Figure 3) vegetational planes is being defined. A finely textured green horizontal vegetational plane best agrees with plant species displaying morphological properties of small shrubs, above-ground growth, spreading habitus, fine foliage texture and green leaf colour.

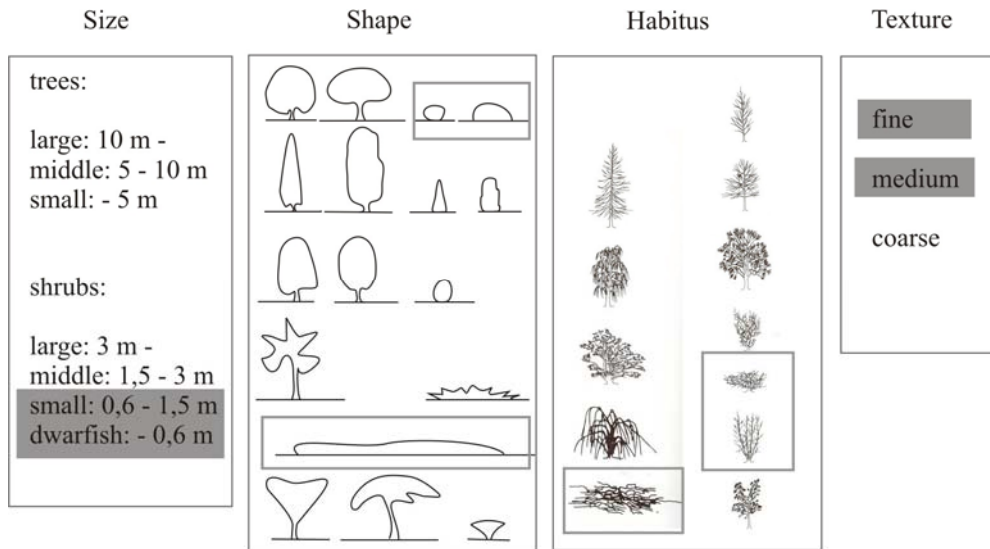


Figure 2. Morphological characteristics of plants used for horizontal vegetation plane

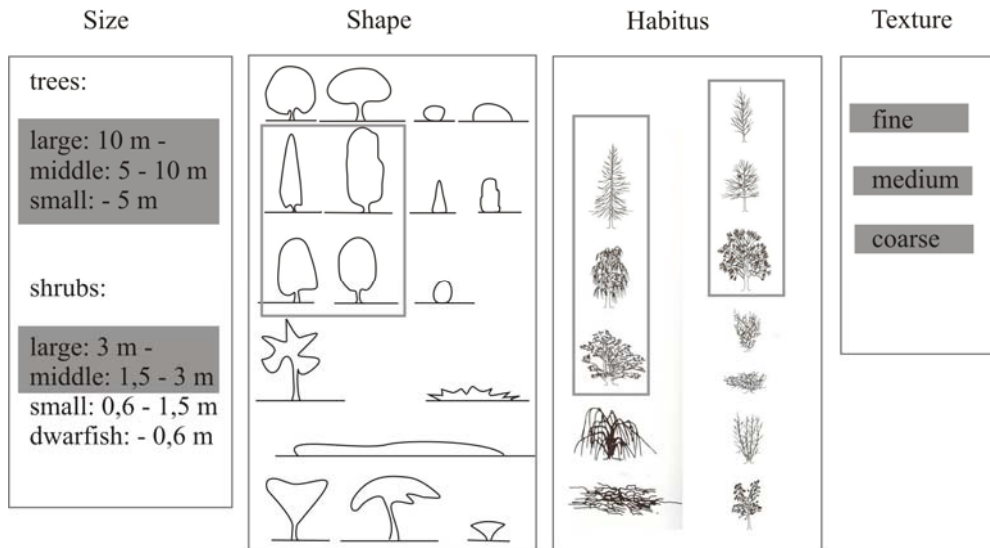


Figure 3. Morphological characteristics of plants used for vertical vegetation plane

The next step is the selection of plant species according to the desired visual effects of the vegetational plane. The selection process is introduced by means of a concrete example and represents one of the results of this research. The suitability of plant species for the construction of vegetational planes is determined according to the following criteria: size, foliage durability, shape, habitus, colour (leaf, blossom, shoot). The criteria are introduced gradually, each added criteria representing a new level or rather combination of morphological properties. We consider the most suitable those species that conform most optimally to all levels of selection and all combinations of criteria.

4 RESULTS AND DISCUSSION

Results of this research are presented in two condensed complexes:

1. Systematizing the plant species according to their morphological properties; this comprehensive part of the results is not shown in the article. It is however of great importance for the process of selection itself. By the same token it represents an autonomously applicable outcome, since the elaborate complex classification enables us to look for adequate plants with regard to their visual properties (size, shape, habitus, texture, colour) and seasonal appearance.
2. The process of plant selection itself in terms of the effect that the plant species is capable of expressing when appearing as a constituent part of a certain vegetational element.

The selection of plant species in terms of desired visual effects of the vegetational plane shall now be demonstrated on a concrete example. Prior to the selection of certain plant species a distinct design goal (or respectively a pre-formulated design request) for a certain visible effect of the landscape element is presented. Thus, for example, by making a certain choice we wish to find the best possible plant species for the construction of a vegetational plane whose visual effects are defined by its height, texture, colour and the point in time when a certain effect will be taking place.

Example: horizontal vegetational plane

The expected properties (design requirements): height 60-150 cm, uniform, homogenous, compact; upper surface modulated in the shape of low clumps, small heaps (clumpy structure), finely textured both in the leafy and the leafless state, of green colour in the period of vegetation, shoots of a distinct colour during winter time.

Stipulating morphological properties of adequate plant species:

- size: *shrub (60 -150 cm)*
- foliage durability: *deciduous*
- shape: *rounded to broadly spreading*
- habitus: *densly packed mass*
- texture - leaf: *fine*
- colour - leaf: *green*
- colour – shoot: *intensive*

Definition of criteria for selection and a list of species that conform to these criteria:

1. Size - foliage durability - habitus

<i>Caryopteris incana</i>	<i>Rhamnus frangula</i>
<i>Cytisus praecox</i> 'Hollandia'	<i>Ribes alpinum</i>
<i>Cytisus scoparius</i>	<i>Salix helvetica</i>
<i>Cytisus x praecox</i>	<i>Salix purpurea</i> 'Nana'
<i>Cytisus x praecox</i> 'Allgold'	<i>Salix rosmarinifolia</i>
<i>Genista tinctoria</i>	<i>Spiraea japonica</i> 'Goldflame'
<i>Prunus tenella</i> 'Fire Hill'	<i>Spiraea japonica</i> 'Litle Princess'
<i>Prunus triloba</i>	<i>Spiraea x bumalda</i> 'Anthony Waterer'

2. Size - foliage durability - habitus – shape

<i>Caryopteris incana</i>	<i>Salix helvetica</i>
<i>Cytisus praecox</i> 'Hollandia'	<i>Salix purpurea</i> 'Nana'
<i>Cytisus scoparius</i>	<i>Salix rosmarinifolia</i>
<i>Cytisus x praecox</i>	<i>Spiraea japonica</i> 'Goldflame'
<i>Cytisus x praecox</i> 'Allgold'	<i>Spiraea japonica</i> 'Little Princess'
<i>Genista tinctoria</i>	<i>Spiraea x bumalda</i> 'Anthony Waterer'
<i>Ribes alpinum</i>	

3. Size - foliage durability - habitus – shape- texture

<i>Caryopteris incana</i>	<i>Salix purpurea</i> 'Nana'
<i>Cytisus praecox</i> 'Hollandia'	<i>Salix rosmarinifolia</i>
<i>Cytisus scoparius</i>	<i>Spiraea x bumalda</i> 'Goldflame'
<i>Cytisus x praecox</i>	<i>Spiraea japonica</i> 'Little Princess'
<i>Cytisus x praecox</i> 'Allgold'	<i>Spiraea x bumalda</i> 'Anthony Waterer'
<i>Genista tinctoria</i>	

4. Size - foliage durability - habitus – shape – texture – color (shoots, leaves)

<i>Cytisus praecox</i> 'Hollandia'
<i>Cytisus scoparius</i>
<i>Cytisus x praecox</i>
<i>Cytisus x praecox</i> 'Allgold'
<i>Genista tinctoria</i>

The last five chosen plant species represent the result of the selection and conform to all selection criteria. The choice of plants is adequate in terms of desired effects of the horizontal vegetational plane - yet sparse. The differences among the species appear in terms of their capacity to modulate the surface (clump structure). When choosing suitable species, adequate for the creation of a certain visible effect of a horizontal vegetational plane (the example of choice here shown) the most important property to be considered is the *size* of the shrubs and the way they grow – *habitus* (before *shape*). Therefore in selection criteria *habitus* comes before *shape*. The most suitable is *Genista tinctoria* (compact shrubs, tightly packet stems form a low clump). *Cytisus praecox* 'Hollandia' in *C. x praecox* 'Allgold' are somewhat taller, the shoots are longer and ascend, and therefore show some difficulty in intertwining, their clump structure is less pronounced.

The species *Cytisus scoparius* presents the greatest difficulty in how to predict the form of its growth. It is nevertheless suitable for the construction of vegetational planes on larger surfaces (it is a good ground-cover plant to be used for the greening in the landscape). For all the enumerated species it is imperative to consider their demands for heat, good lighting as well as the soil reaction.

5 CONCLUSION

The method used and here described for the selection of plant species represents a novelty in the field of landscape design. By means of this research it was ascertained that the manner of selecting plants according to their visual properties is appropriate. For a final selection it is necessary to add to the morphological criteria some other criteria, which are

formulated on the basis of eco-physiological needs of each plant (lighting, temperature, soil conditions) and technological demands (cultivation requirements).

Therefore it is imperative that we emphasize the relativity of such plant selection, as is evident from the above commentary, relating to a concrete example of choosing appropriate plants.

The results of the research are applicable on different levels. By using the data gained in the research (systemizing the plant species according to their visual properties) we can find species that belong to certain size classes, shapes, colours, and textures.

The data can be combined arbitrarily in order to search for cross-section multitudes that in essence represent combinations of morphological properties.

The data and the above mentioned systematizations are not presented in this paper, due to the amplitude / extensiveness of the material.

Alongside the selection criteria as such it is also important in which order or rather sequence the criteria, upon which we base the selection of the plants, are applied. Which criteria are to be included and in what order, largely depends on the design goal. The paper demonstrates the process of selection of plant species suitable for horizontal vegetational planes. In a similar way suitable plants for other landscape elements can be chosen (line, point, clump and similar).

The presented process of selection is not applicable only in landscape architecture. It can be equally useful in selective breeding, since it can help determine the need for a particular shape, habitus, colour ... In terms of its informative quality it can be applied by tree planters, horticultural experts and may also be seen as a welcome aid in the education of landscape architects and horticultural experts.

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Agrovoc descriptors: plants; transpiration; gas exchange; physiological functions; carbon dioxide; evaporation; stomata

Agris category code: F40, F50, F61

COBISS koda 1.02

Regulacija prevodnosti listnih rež

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IZVLEČEK

Kopenske rastline se soočajo s problemom, kako sprejeti iz ozračja ustrezno količino CO₂ in pri tem omejiti oddajanje vode. Rešitev tega problema je uravnavanje prevodnosti listnih rež. Da je v danih razmerah dosežena optimalna prevodnost rež, se celice zapiralke odzivajo na mnoge signale iz okolja in na signale iz rastline. V članku predstavljamo odzive listnih rež na glavne abiotske dejavnike in pri prikazu učinkov teh dejavnikov uporabimo lastne rezultate porometričnih meritev. Opisan je prenos signalov v regulaciji listnih rež, v opisu so vključene nekatere nedavne ugotovitve na tem področju.

Ključne besede: listne reže, prevodnost listnih rež, regulacija, signalna mreža, izmenjava plinov, transpiracija

ABSTRACT

REGULATION OF STOMATAL CONDUCTIVITY

Land plants are faced with competing demands to take up CO₂ from the atmosphere while limiting water loss. The functional solution to this dilemma is the regulation of stomatal apertures. The guard cells of stomata respond, in order to achieve optimal conductance, to various environmental and endogenous stimuli. In this paper the effects of main abiotic factors on stomata are described and presented by original porometric data. In addition the signal transduction in the regulation of guard cells response, including recent findings related to this topic is discussed.

Key words: stomata, stomatal conductivity, regulation, signal network, gases exchange, transpiration

1 UVOD

Preproste rastline, ki vodne bilance ne uravnavajo aktivno, vodo hitro oddajajo skozi svojo površino, preko katere izmenjujejo tudi pline, ki sodelujejo v rastlinski

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presnovi. Imenujemo jih poikilohidre rastline in so vezane na rastišča z visoko vlažnostjo. S prehodom rastlin na kopno je postala dostopnost vode najpogostejši omejujoči dejavnik rasti in razvoja. Rastline so v odgovor na ta selekcijski pritisk razvile tkiva in organe, ki jim omogočajo učinkovito preskrbo z vodo (korenine in prevodna tkiva) in tkiva, ki omejujejo izgubo le-te (krovnna tkiva s svojimi diferenciacijami). Kutikula na površini nadzemnih organov višjih kopenskih rastlin lahko močno omeji izgubo vode, a hkrati tudi ovira izmenjavo plinov, ki jih rastlina potrebuje (CO_2 , O_2). Za učinkovito izmenjavo plinov in nadzorovano oddajanje vode so rastline v krovnih tkivih razvile posebne aktivno regulirane odprtine – reže (lat. stomata). Reže se lahko pojavljajo na različnih delih rastline, vendar v največjem obsegu na listih (listne reže). Izmenjava plinov skozi reže poteka z difuzijo, transportom, pri katerem je gonilna sila razlika v koncentraciji plina med atmosfero in rastlino. Koncentracijski gradient je poleg upornosti, ki jo plin srečuje na difuzijski poti, odločilen dejavnik hitrosti difuzijskega toka. Izrazimo ga lahko tudi kot razliko parcialnega tlaka vodne pare atmosfere in lista (deficit tlaka vodne pare). Poleg celičnih mehanizmov, npr. uravnavanja vodnega statusa celice s pomočjo osmoregulacije, predstavljajo reže osnovo za aktivno uravnavanje vodne bilance, ki jo poznamo pri homojohidrih rastlinah.

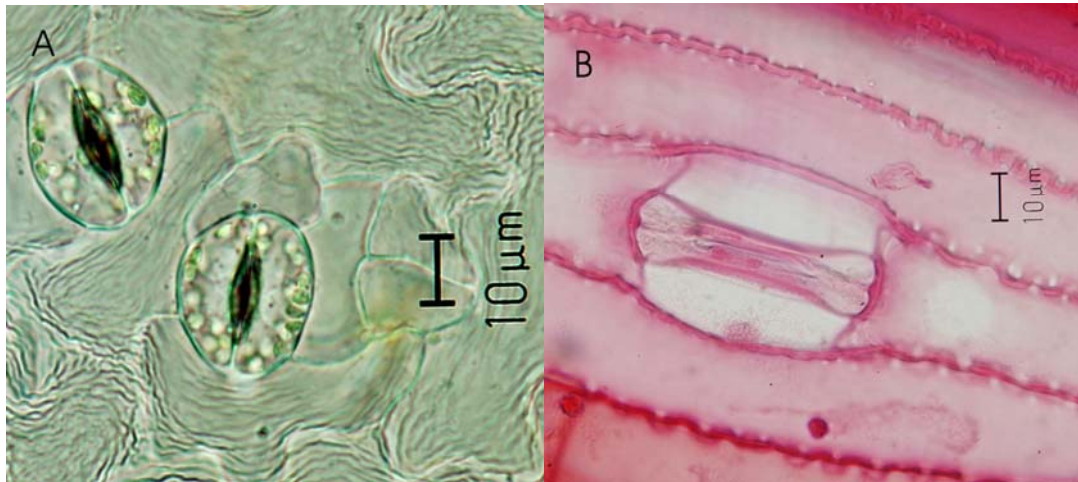
Rastlina običajno, ko ni potrebe po izmenjavi drugih plinov, omejuje izgubo vode z zapiranjem listnih rež. Večina rastlin pa mora v dnevnem času, ko v zelenih tkivih poteka fotosinteza, zagotoviti sprejem CO_2 iz okoliškega zraka. Da zagotovi nemoten potek fotosinteze, rastlina odpre reže, s čimer pa se pospeši tudi proces oddajanja vode (transpiracija). Potrebno je torej kontrolirano uravnavanje prevodnosti listnih rež, ki je vedno sprejemanje kompromisa, kako zadostiti presnovnim potrebam v okviru fotosintezne vezave atmosferskega ogljika in kako hkrati s primernimi omejitvami transpiracije ohraniti pozitivno vodno bilanco rastline. Ta odnos med fotosintezo in transpiracijo opredelimo s parametrom, ki ga imenujemo učinkovitost izrabe vode (water use efficiency).

Rastline so, da je dosežena optimalna izmenjava plinov ob čim manjši izgubi vode, razvile zapleten sistem regulacije odprtosti rež. Listne reže se odzivajo na mnoge okoljske in notranje (endogene) signale, na vsakega na določen način. Različni dejavniki pa nikoli ne delujejo ločeno, temveč se njihovi vplivi prepletajo. Prav tako pa so med seboj prepletene signalne poti, preko katerih dejavniki sprožajo spremembe prevodnosti rež. Prav zapletena signalna mreža omogoča rastlinam doseganje za dane razmere optimalne odprtosti listnih rež in prej omenjenega kompromisa. V tem članku kratko predstavljamo vplive glavnih dejavnikov, ki določajo prevodnost listnih rež in dosedanja spoznanja o delovanju mrež pri prenosu signalov.

2 ZGRADBA LISTNIH REŽ

Aktivni del listne reže sta dve celici zapiralki (ang.: guard cell), med katerima se nahaja odprtina. Celici zapiralki lahko spremljata celici spremljevalki ali pa sta obdani z običajnimi celicami povrhnjice (slika 1). Celice zapiralke s spremembo koncentracije osmotsko aktivnih snovi uravnavajo vsebnost vode, kar se odraža na njihovem volumnu in znotrajceličnem tlaku (turgorju, potencialu tlaka). Urejena razporeditev mikrofibril celuloze v celičnih stenah določa, v katere smeri se lahko širi celica, ko se ji poveča volumen. Pri listnih režah radialna struktura v celični steni

povzročijo, da se celici zapiralki ob povečanju turgorja razmakneta in tako oblikujeta režo. Običajno je reža polkrožna (slika 1a), pri graminejskem tipu listnih rež, ki se pojavlja pri travah in šaših, pa je bolj podolgovata (slika 1b).



Slika 1a, b: Mikroskopske slike listnih rež. A: Dva kompleksa listnih rež v povrhnjici ciklame (*Cyclamen persicum* Mill.). Po dve celici zapiralki sta obdani z običajnimi celicami povrhnjice. Slika je narejena na sveže odluščeni povrhnjici. B: Kompleks listne reže trave bilnice (*Festuca* sp.). Vidna je značilna oblika graminejskega tipa celic zapiralk s podolgovato listno režo med njima. Celici zapiralki sta obdani s celicama spremljevalkama (svetlo), okoli pa so podolgovate celice listne povrhnjice. Na sliki je listna povrhnjica, s katere je bilo ostalo tkivo odstranjeno s strganjem (Fotografiji: B. Turk).

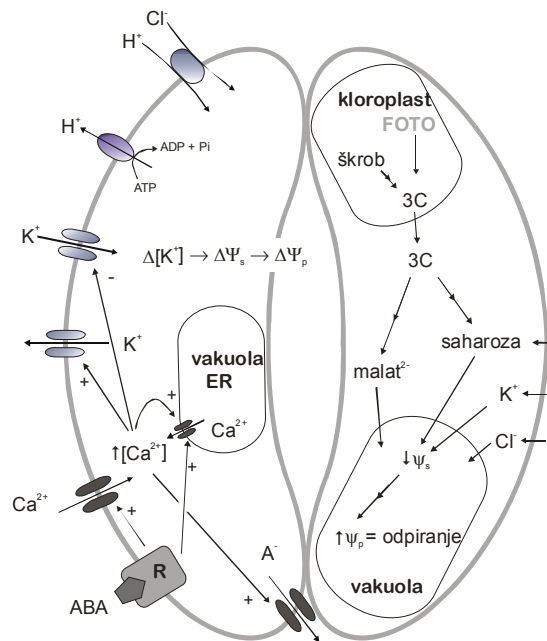
Figure 1a, b: Microscopic picture of stomata. A: Two stomatal complexes in cyclamen (*Cyclamen persicum* Mill.) epidermis. Two guard cells are surrounded with epidermal cells. Picture was taken on fresh epidermal peel. B: Stomatal complex of fescue grass (*Festuca* sp.). Typical graminoid stomata with elongated guard cell can be seen. Two guard cells are accompanied with two subsidiary cells (bright). Specimen was prepared as leaf epidermal slice, lower layers of cells were removed from covering tissue by scraping (Photo: B. Turk).

3 MEHANIZEM DELOVANJA LISTNIH REŽ

Kot je bilo že omenjeno, listne reže uravnavajo svojo odprtost z uravnavanjem koncentracije osmotsko aktivnih snovi in posledično količine vode v celici (slika 2). Razpoložljivost vode v rastlinski fiziologiji opisujemo z vodnim potencialom (ψ_w , enota MPa), ki vključuje različne dejavnike, ki vplivajo na razpoložljivost vode v rastlini. Skupni vodni potencial ψ_w sestavljajo prispevki osmotskega potenciala (ψ_s , odvisen od količine raztopljenih snovi v vodi), potenciala tlaka (ψ_p), gravitacijskega potenciala (ψ_g , enak potencialni energiji vode) in matričnega potenciala (ψ_m , v primerih, ko je razpoložljivost vode zmanjšana zaradi interakcij molekul vode s površino). V protoplastu rastlinske celice sta glavni komponenti vodnega potenciala negativen osmotski potencial in pozitiven potencial tlaka (turgor), običajno se skupni vodni potencial rastlinske celice giblje med -0. in -3 MPa (referenčni vodni potencial $\psi_w = 0$ MPa ima voda brez topljencev pri okoljskem tlaku in temperaturi).

Voda bo prehajala v celico zapiralko, kadar bo njen vodni potencial manjši od vodnega potenciala v okolici. Takšno stanje je doseženo, kadar se v celici zapiralki oz. njeni vakuoli nakopičijo osmotsko aktivni topljenci, s čimer se zmanjša osmotski potencial (ψ_s) in s tem skupni vodni potencial (ψ_w) celice zapiralke.

Osnova za hitre spremembe osmotskega potenciala je prehajanje kalijevih ionov v celico zapiralko in iz nje (slika 2, levo). Gre za difuzijo ionov K^+ skozi ionske kanalčke dveh tipov, enim za vstop K^+ v celico (K^+_v) in drugih za izhajanje K^+ iz celice (K^+_iz). Vstop K^+ je možen, kadar je na membrani s pomočjo protonskih črpalk (H^+ -ATPaze) ustvarjen elektrokemijski gradient, ki predstavlja energijo in signal za nadaljnje transportne procese. Hkrati z vstopom K^+ v celico vstopajo tudi kloridni anioni za potrebe uravnavanja razmerja kationi/anioni v celici. Slednje se lahko uravnava tudi s pomočjo organskih anionov ($malat^{2-}$), ki jih tvori celica zapiralka v presnovi ogljikovih hidratov. Presnova ogljikovih hidratov je pomembna tudi pri sintezi organskih osmotikov npr. pri nastanku osmotsko aktivne saharoze iz osmotsko neaktivnega škroba (slika 2, desno). Saharoza lahko preko dneva nadomesti K^+ in vzdržuje majhen osmotski potencial in s tem velik potencial tlaka v času, ko mora biti listna reža odprta. Ob zapiranju rež se opisani dogodki vršijo v obrnjenem sledju.



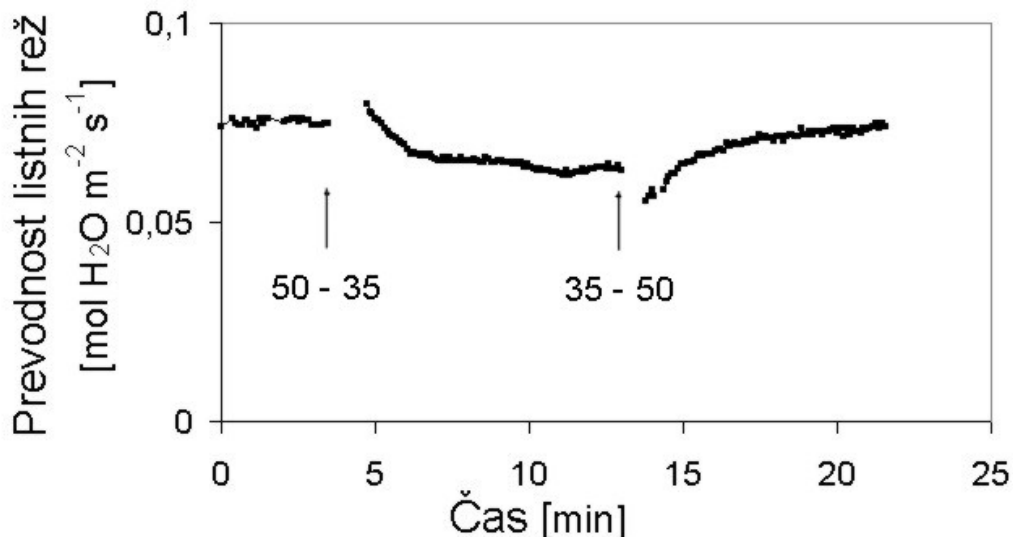
Slika 2: Levo: Različni membranski transportni proteini na celični membrani celic zapiralk. Delovanje kanalčkov in črpalk je ključno za spremembo količine K^+ v celici, osmotskega potenciala (ψ_s) in s tem potenciala tlaka (ψ_p) v zapiralkah. Glavno osmotsko funkcijo vrši vakuola, zato so podobni transportni mehanizmi prisotni tudi na njeni membrani (tonoplastu), na sliki pa niso prikazani. V spodnjem delu leve celice je prikazana signalna vloga Ca^{2+} pri zapiranju listnih rež v odziv na ABA. Desno: Osmotsko funkcijo K^+ lahko čez dan prevzame saharoza, katere izvor je škrob, neposredno fotosinteza ali pa saharoza prihaja iz sosednjih celic z aktivnim transportom. Škrob služi tudi kot vir organskih anionov ($malat^{2-}$), ki uravnovežijo naboj (K^+) v vakuoli. Isto funkcijo lahko vrši tudi kloridni anion (Cl^-), za katerega je kotransport v celico prikazan na levi celici zgoraj. (A^- - anioni, ER - endoplazmatski retikulum, Δ - sprememba, FOTO - fotosinteza, ABA - abscizinska kislina, R - receptor).

Figure 2: Left: Different transport proteins on the plasma membrane of the guard cells, e.g. channels and pumps, are crucial for the changes of potassium concentration and the consequent changes in osmotic (ψ_s) and pressure (ψ_p) potentials. The main osmotic function is performed by vacuoles, therefore similar transport mechanisms are present also on tonoplast (not shown on this figure). On the lower part of the cell we present the signal transduction mediated by changes of Ca^{2+} concentrations after ABA sensing. Right: During the day osmotic function of K^+ is overtaken by sucrose. Sucrose may originate from starch degradation, from photosynthesis or may enter the cell from neighboring cells by active transport. Starch may act also as a source of organic anions ($malate^{2-}$), which counterbalance cations (K^+) in vacuoles. The same function can be performed also by Cl^- . The cotransport of Cl^- into

the guard cells is presented on the upper part of the left cell. (A^- - anions, ER - endoplazmatic reticulum, Δ - change, FOTO - photosynthesis, ABA - abscisic acid on plasma membrane receptor)

4 ODZIVI LISTNIH REŽ NA SPREMEMBE NEKATERIH ABIOTSKIH DEJAVNIKOV

Kot omenjeno je najpogostejši omejujoč dejavnik rasti in razvoja višjih kopenskih rastlin pomanjkanje vode. Regulacija odprtosti listnih rež pri odzivu na spremembo vodnega potenciala lista je v določeni meri neposredna. Ko se zniža vsebnost vode v listu, se zniža tudi vsebnost vode v celicah zapiralkah, kar pomeni, da pade turgor, zaradi česar se listne reže zaprejo. Listne reže se, s tem da po do sedaj nepojasnjem mehanizmu zaznavajo obseg transpiracije (Buckley in sod. 2005), odzivajo tudi na spremembe vodnega potenciala atmosfere. Če je okoliški zrak bolj suh se priprejo (slika 3) in tako preprečijo, da bi vsebnost vode v listu preveč padla. Pri daljšem trajajočem pomanjkanju vode je njihovo zapiranje uravnano z abscizinsko kislino (ABA), katere vsebnost v listih ob suši močno naraste.

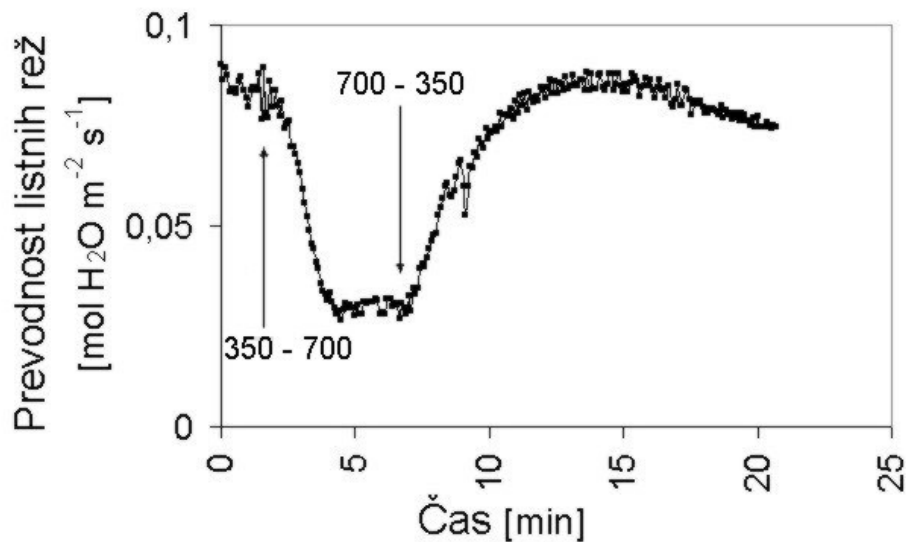


Slika 3: Odziv listnih rež koruze (*Zea mays* L.) na spremembo relativne vlažnosti okoliškega zraka. Puščici in spremljajoče številke označujejo čas in obseg spremembe relativne vlažnosti v %. Presledek v liniji prevodnosti listnih rež predstavlja čas, ko zaradi načina meritve ne moremo slediti odzivu listnih rež. Odziv smo merili z merilnim sistemom Li-6400 (LICOR, Lincoln, ZDA) pri 25°C, jakosti svetlobe 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in 400 $\mu\text{mol CO}_2 \text{mol}^{-1}$. Podatki so bili beleženi v intervalu 5 sekund.

Figure 3: Stomatal response of maize (*Zea mays* L.) to the changes of relative humidity. Arrows and accompanying numbers indicate time and the level [%] of relative humidity change. The breaks in the curve result from the fact that with the technique applied, it is not possible to follow stomatal conductivity shortly after the change in RH. Stomatal responses was measured with Li-6400 system (LICOR, Lincoln, USA) at 25°C, 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity and 400 $\mu\text{mol CO}_2 \text{mol}^{-1}$. Data were logged in 5 second intervals.

Na odprtost listnih rež vpliva tudi **koncentracija CO_2** ($[\text{CO}_2]$). Listne reže zaznavajo $[\text{CO}_2]$ v zraku znotraj lista (C_i - intercelularna $[\text{CO}_2]$). Fotosinteza porablja CO_2 v listu. Znižana C_i je signal za odpiranje listnih rež, saj si rastline z večjo prevodnostjo rež zagotovijo dovolj CO_2 za fotosintezo. Ko pa C_i zadosti naraste, kar je lahko posledica zadostne odprtosti listnih rež ali pa prevlade dihanja nad fotosintezo ob pomanjkanju svetlobe, se listne reže zaprejo in s tem omejijo transpiracijo (slika 4). V poskusu prikazanem na sliki 4 smo list koruze med meritvijo izpostavili 350 oz. 700

$\mu\text{mol mol}^{-1} \text{CO}_2$. Ob povečanju koncentracije se je C_i spremenil iz 150 na 250 $\mu\text{mol mol}^{-1}$, kar nakazuje, da rastlina uravnava $[\text{CO}_2]$ znotraj lista. O tem, kako listne reže zaznavajo C_i in kako poteka prenos signala do odziva listnih rež, obstaja še veliko nejasnosti. Trenutno potekajo intenzivne znanstvene diskusije o pomenu fotosinteze pri zaznavanju C_i (glej točko 5; Messinger in sod., 2006; Israelsson in sod., 2006).



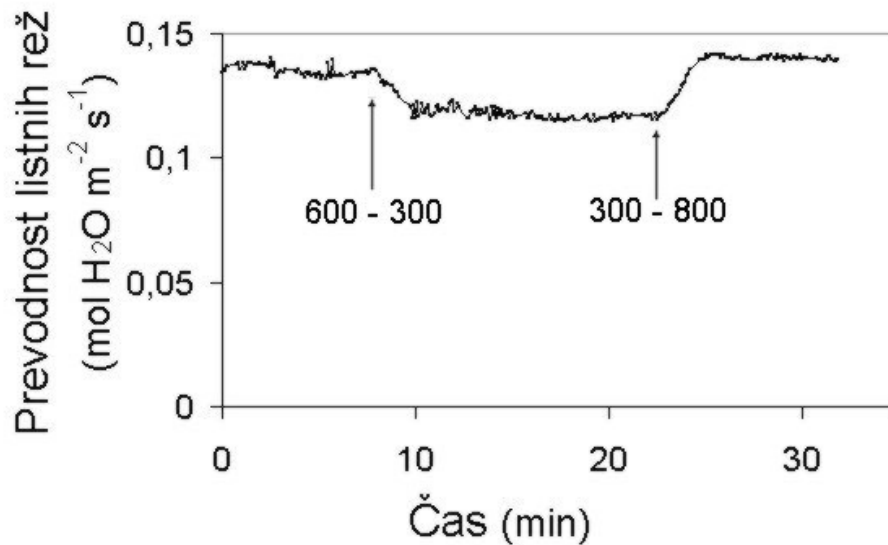
Slika 4: Odziv listnih rež koruze (*Zea mays* L.) na spremembo $[\text{CO}_2]$ v okoliškem zraku. Prikazan je odziv listnih rež na spremembo $[\text{CO}_2]$. Puščice in spremljajoče številke označujejo čas in obseg spremembe $[\text{CO}_2]$ v $\mu\text{mol mol}^{-1}$. Odziv smo merili z merilnim sistemom Li-6400 (LICOR, Lincoln, ZDA) pri 26°C, jakosti svetlobe 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in relativni vlažnosti 35%. Podatki so bili beleženi v intervalu 5 sekund.

Figure 4: Stomatal response of maize (*Zea mays* L.) to the changes of $[\text{CO}_2]$ in surrounding air. Arrows and accompanying numbers indicate time and the level $[\mu\text{mol CO}_2 \text{mol}^{-1}]$ of CO_2 concentration change. Stomatal response was measured with Li-6400 system (LICOR, Lincoln, USA) at 26°C, 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity and relative humidity of 35%. Data were logged in 5 second intervals

Podobno kot pri C_i je tudi odziv listnih rež na **svetlobo** v določeni meri povezan s fotosintezo. Na prevodnost rež lahko vpliva tako fotosinteza znotraj lista (v mezofilu) kot fotosinteza v samih celicah zapiralkah. Svetloba v določeni meri vpliva preko porabe CO_2 , kar nakazuje naša meritev, ki je prikazana na sliki 5, pri kateri je rastlina ohranila C_i skoraj nespremenjen. Obstajajo pa dokazi o neposrednem vplivu svetlobe (modra svetloba) na odprtost listnih rež.

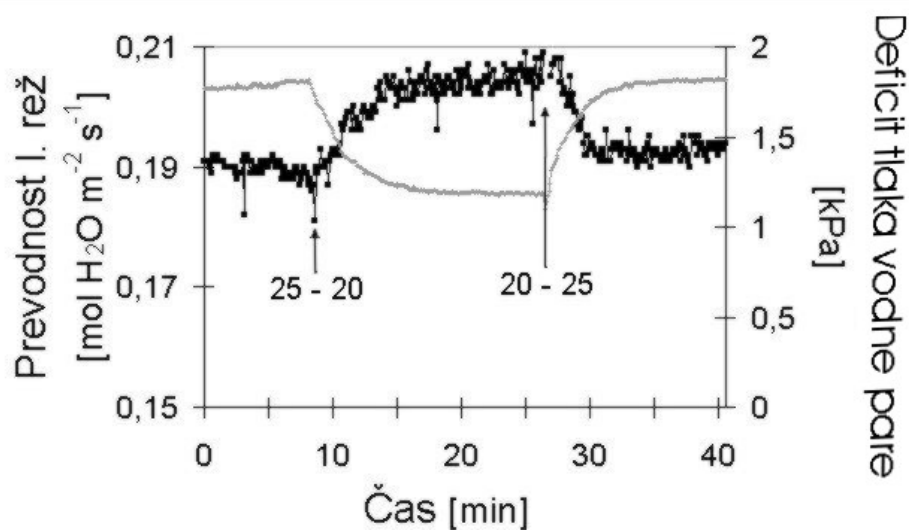
Modra svetloba vpliva na mnogo fizioloških procesov v rastlinah. Zaustavlja podaljševanje stebela, povzroča fototropizem in vpliva na izražanje genov. Prav tako modra svetloba spodbuja odpiranje listnih rež. Odkrili so tudi, da zelena svetloba deluje nasproti vplivu modre svetlobe. Če listne reže osvetlimo s pulzom modre svetlobe, zatem pa še s pulzom zelene svetlobe, ne pride do siceršnjega odziva odpiranja listnih rež (Taiz in Zeiger, 2006).

Pomemben okoljski dejavnik je tudi **temperatura**. Rastline ob nespremenjenih ostalih okoljskih vplivih nad določeno temperaturo odpirajo listne reže in se s transpiracijo hladijo. Tako povečano transpiracijo pa začnejo listne reže ponovno omejevati, ko pade vodni potencial v listu. Na sliki 6 je prikazan odziv listnih rež na spremembo temperature. Pri njem pa ob uporabljenem načinu meritve težko izločimo vpliv padca relativne vlažnosti, ki nastane ob dvigu temperature.



Slika 5: Odziv listnih rež koruze (*Zea mays* L.) na spremembo jakosti svetlobe. Puščici in spremljajoče številke označujejo čas in obseg spremembe jakosti svetlobe v $\mu\text{mol m}^{-2} \text{s}^{-1}$. Odziv smo merili z merilnim sistemom Li-6400 (LICOR, Lincoln, ZDA pri 26°C , $350 \mu\text{mol CO}_2 \text{mol}^{-1}$ in relativni vlažnosti 35%. Podatki so bili beleženi v intervalu 5 sekund.

Figure 5: The stomatal response of maize (*Zea mays* L.) to changes in light intensity. Arrows and accompanying number indicate time and the level [$\mu\text{mol m}^{-2} \text{s}^{-1}$] of light intensity change. Stomatal responses was measured with Li-6400 system (LICOR, Lincoln, USA) at 26°C , $350 \mu\text{mol CO}_2 \text{mol}^{-1}$ and relative humidity of 35%. Data were logged in 5 second intervals.



Slika 6: Odziv listnih rež osleza (*Hibiscus* sp.) na spremembo temperature (črno). Puščici in spremljajoče številke označujeta čas in obseg spremembe temperature v $^{\circ}\text{C}$. Prikazan je tudi deficit tlaka vodne pare (sivo), ki nam pove kolikšna je gonilna sila transpiracije. Ob znižanju temperature se je znižal tudi deficit vodne pare, kar bi lahko tudi vplivalo na regulacijo listnih rež (kot v poizkusu predstavljenim na sliki 3). Odziv smo merili pri $15 \mu\text{mol H}_2\text{O mol}^{-1}$, $350 \mu\text{mol CO}_2 \text{mol}^{-1}$ in $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ svetlobe. Podatki so bili beleženi v intervalu 5 sekund.

Figure 6: The stomatal response of rosemallow (*Hibiscus* sp.) to temperature changes (black). Arrows and accompanying numbers indicate time and the level [$^{\circ}$] of temperature change. The level of water vapour pressure deficit, a transpiration driving force, is also presented (in grey). By lowering the temperature water pressure deficit also decreased what can also influence stomatal regulation (similar to experiment presented on figure 2). Responses was measured with Li-6400 system (LICOR, Lincoln, USA) at $15 \mu\text{mol H}_2\text{O mol}^{-1}$, $350 \mu\text{mol CO}_2 \text{mol}^{-1}$ and light intensity $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$. Data were logged in 5 second intervals.

Na odprtost listnih rež vplivajo tudi endogeni dejavniki kot so hormoni in druge molekule, ki lahko prinašajo signale iz drugih delov rastline. Med hormoni ima najpomembnejšo signalno vlogo abscizinska kislina. Izvira lahko iz različnih tkiv rastline. Ob suši nastaja v koreninah in se sprošča tudi iz celičnih rezerv v drugih tkivih npr. v listu. Ko se njena koncentracija poveča se listne reže zaprejo. Avksini na drugi strani spodbujajo odpiranje listnih rež.

5 PRENOS SIGNALOV

V prejšnji točki so bili na kratko predstavljeni nekateri signali, ki vplivajo na spremembe prevodnosti listnih rež. Pot od zaznave nekega abiotskega ali biotskega vpliva do odgovora celic zapiralk poteka preko signalnih verig, ki so sestavljene iz več signalnih dogodkov. Proučevanje prenosa signala v celicah zapiralkah je precej zapleteno, saj se signalne poti za posamezne vplive prepletajo, ob hkratnem delovanju različnih dejavnikov. Prav zapletena signalizacija pa je osnova za to, da lahko rastlina v določenih razmerah učinkovito poskrbi za primerno prevodnost listnih rež.

Brez dvoma je največ poznanega o prenosu signala abscizinske kisline (ABA), hormona, katerega koncentracija v listih ob sušnem stresu močno naraste. ABA vpliva ne samo na listne reže, ampak tudi na druge dogodke, ki so del odgovora rastlin na pomanjkanje vode. V primeru celic zapiralk so različni poskusi pokazali, da so lahko receptorji za zaznavanje abscizinske kisline tako zunajcelični, torej na plazmalemi (ABAP1, Razem in sod., 2006; slika 2), kot tudi znotrajcelični. Prvi naj bi regulirali predvsem preprečevanje odpiranja listnih rež in ekspresijo genov, drugi pa inducirali zapiranje listnih rež ter inhibirali tiste kanalčke, katerih funkcija je pomembna za odprtje reže. Prenos signala ABA v celici zapiralki poteka po več poteh, od katerih večina vključuje Ca^{2+} kot sekundarni sporočevalec. Koncentracija tega iona v citosolu ($[\text{Ca}^{2+}]_c$) naraste potem, ko se zaradi ABA odprejo kanalčki za vstop Ca^{2+} v citosol iz okolice celice (apoplast) ali iz znotrajceličnih rezerv (ER, vakuola) (slika 2). Pri tem lahko kot posredniki med kompleksom ABA-receptor in kanalčki delujejo reaktivne kisikove zvrsti (ROS), inozitolfosfati, ciklična ADP-riboza, NO in druge signalne molekule (Schroeder in sod., 2001). Različni poskusi so pokazali, da povečana koncentracija $[\text{Ca}^{2+}]_c$ blokira K^+_v kanalčke, zavira delovanje protonskih črpalk (H^+ -ATPaze) ter aktivira kanalčke K^+_{iz} . Skupen rezultat teh procesov pa je depolarizacija celične membrane, izstop ionov in zapiranje listnih rež. Signalizacija preko Ca^{2+} pa je še veliko bolj zapletena, kot so nakazovale prve raziskave. Ugotovili so namreč, da so za prenos signala ABA pomembna nihanja $[\text{Ca}^{2+}]_c$. Takšna nihanja so prisotna tudi pri odzivu na nizko koncentracijo CO_2 in avksin, ki pa oba povzročata nasproten učinek, t.j. odpiranje rež. Zelo verjetno je, da je za odgovor celice zapiralke pomembna časovna in prostorska komponenta sprememb $[\text{Ca}^{2+}]_c$ v citosolu (Roelfsema, 2005).

ABA deluje tudi po poteh, ki niso neposredno povezane s povečanjem $[\text{Ca}^{2+}]_c$. Tu gre predvsem za signaliziranje, v katerem sodelujejo nekateri produkti presnove fosfolipidov in encimi fosfataze (Fan in sod. 2004).

Pri proučevanju prenosa signala svetlobe, je osnovna težava v ločevanju med neposrednimi in posrednimi (fotosintezni) vplivi svetlobe na reže. V okviru neposrednih učinkov, je največ raziskav opravljenih za modro svetlobo. Znano je, da modra svetloba na odpiranje reže vpliva z vzpodbujanjem aktivnosti protonskih

črpalk (H^+ -ATPaze). Te črpalke gradijo protonski gradient, ki je osnova za sprejem K^+ skozi K^+_v kanalčke in kotransport Cl^- v celico. V celicah zapiralkah naj bi bil receptor za modro svetlobo zeaksantin, pigment ksantofilnega cikla, ki sicer v kloroplastih služi za odvajanje presežne energije svetlobe. V prid tej hipotezi govorijo dejstva, I) da se absorpcijski spekter zeaksantina dobro ujema z akcijskim spektrom za odpiranje rež v odzivu na modro svetlobo; II) da ima dnevni hod koncentracij zeaksantina dobro korelacijo z dnevnim hodom jakosti osvetlitve in prevodnosti listnih rež; III) da se občutljivost rež na modro svetlobo veča s koncentracijo zeaksantina v celicah zapiralkah in IV) da je moč z blokado sinteze zeaksantina preprečiti značilen odziv rež na modro svetlobo. Hipotezo o pomenu zeaksantina pri odzivu rež lahko podkrepi dejstvo, da je moč z izomerizacijo zeaksantina razložiti pojav modro-zelene reverzibilnosti. To pa ne velja za še dva rastlinska fotoreceptorja, ki tudi zaznavata modro svetlobo (fototropin in kriptokrom) in regulirata druge procese.

Posredni učinki svetlobe na prevodnost listnih rež so v veliki meri povezani s porabo CO_2 v ogljikovih reakcijah fotosinteze. Zmanjšanje C_i je močan signal za odpiranje rež. Ugotovili so, da povečana C_i aktivira počasne anionske kanalčke (S-tip), inhibira K^+_v kanalčke in aktivira K^+_{iz} kanalčke (Fan in sod., 2006), vendar je le malo poznanega o signalni poti pred odzivom ionskih kanalčkov. Povečana C_i praviloma poveča $[Ca^{2+}]_c$ v celici, ta pa zapre listne reže, podobno kot v odzivu na ABA. Kot že omenjeno, sodeluje $[Ca^{2+}]_c$ tudi pri odpiranju listnih rež v odzivu na nizko $[CO_2]$, ob kateri so opazili povečanje frekvence nihanj $[Ca^{2+}]_c$ (Young in sod. 2006). Ob blokadi delovanja $[Ca^{2+}]_c$ se je pokazalo, da se signal CO_2 lahko prenaša tudi z mehanizmom, ki je od $[Ca^{2+}]_c$ neodvisen. Odziv na $[CO_2]$ bi lahko bil uravnavan preko svetlobnih reakcij fotosinteze, na kar sklepajo na podlagi sprememb prevodnosti rež, ki so jih opazili ob blokadi transporta elektronov v svetlobnih reakcijah fotosinteze (Messinger in sod., 2006). Ker svetloba in $[CO_2]$ odločilno vplivata na razmerje med svetlobnimi in ogljikovimi reakcijami fotosinteze, je takšen način regulacije moč pričakovati za oba dejavnika.

Temperatura vpliva na skoraj vse encimske procese v celicah in s tem lahko neposredno ali posredno vpliva tudi na odprtost listnih rež. Ena od možnih razlag vpliva temperature, bi lahko bili različni temperaturni optimumi K^+_v in K^+_{iz} kanalčkov (Ilan in sod., 1995). Tako naj bi sprejem pri višjih temperaturah (nad $20^\circ C$) prevladoval nad izpustom K^+ , kar pomeni odpiranje listnih rež. Ta signal pa izgubi prevlado v regulaciji prevodnosti rež, ko pade vsebnost vode v listu, saj ima rastlina le omejeno sposobnost pridobivanja in prevajanja vode.

6 ZAKLJUČKI

V tem članku smo na kratko orisali osnovne dogodke v regulaciji prevodnosti listnih rež rastlin. Za bolj natančen pregled poznanih in predpostavljenih signalnih poti priporočamo pregledne članke kot na primer: Schroeder in sod. (2001) in Fan in sod. (2004) ali pa knjigo Plant Physiology (Taiz in Zeiger, 2006)

Najbolj raziskana je signalizacija ABA in kaže, da je tudi najbolj zapletena od predstavljenih signalov. Do sedaj so dokazali, da igra v signalni verigi odziva listnih rež na ABA pomembno vlogo vsaj deset encimov, ki tvorijo več bolj ali manj

povezanih verig. Vsaj toliko je še različnih molekul, ki opravljajo nalogo sekundarnih sporočevalcev, ter dogodkov, ki so že del odziva listnih rež na signal ABA (Li in sod. 2006). Z mutanti so odkrili tudi mnoge gene oz. njihove produkte, ki se vključujejo v regulacijo in imajo pozitiven ali pa negativen vpliv na končni odziv listnih rež. Čeprav so za vse te člene pokazali »pomembno« vlogo pri prenosu signala, je težko določiti pomen posameznega člena ali dela verige pri odzivu listnih rež. Obstaja tudi možnost, da imajo različne signalne verige v različnih rastlinskih vrstah različen pomen.

Poleg prenosa signala ABA, ki so ga poizkusili ločeno modelirati Li in sod. (2006), imamo še druge, v tem članku omenjene signale, na katerih je bilo opravljenih manj raziskav. Nekaj raziskav nakazuje, da bi lahko na odzivnost listnih rež vplivali še drugi procesi, kot na primer izgradnja in razgradnja sestavin celične stene (Jones in sod., 2003).

Ločeno raziskovanje posameznih signalov in odzivov nanje je oteženo že zaradi njihove medsebojne povezanosti. Takšen primer je tudi naš poskus prikaza odziva listnih rež na spremembo temperature (slika 6). Kljub ustaljeni absolutni vlažnosti zraka med poskusom, se ob spremembi temperature zraka spremeni relativna vlažnost zraka in s tem deficit tlaka vodne pare, ki lahko kot gonilna sila transpiracije vpliva na odziv listnih rež. S takšnim načinom meritev torej težko oddvojimo vpliv temperature od vpliva relativne vlažnosti zraka. V naravi pa je preplet posameznih signalov še toliko bolj pogost. Npr. v poletni vročini je pogosto za rastline še bolj kot visoka temperatura problem pomanjkanje vode. Ko slednje povzroči zapiranje listnih rež, pride zaradi pomanjkanja CO₂ do s tem povezanega zaviranja fotosinteze. Rastline pa lahko v takšnem stanju doživijo stres zaradi presežne svetlobe, ki je ne morejo koristiti v fotosinteznih reakcijah. Listne reže s pomočjo prepletenih poti združujejo vse te signale in s pomočjo zapletene regulacije v danih razmerah poskušajo vzpostaviti optimalno prevodnost listnih rež.

Obstajajo dokazi, da se odziv listnih rež aklimatizira na določen signal. Talbott in sod. (2003) so na primer ugotovili, da so se rastline, ki so rasle pri večji vlažnosti, hitreje odzivale na spremembe [CO₂]. Ta odzivnost se je pokazala kot reverzibilna. Tudi nekatere naše meritve kažejo, da se listne reže na določen signal kratkoročno aklimatizirajo (Hladnik, neobjavljeni podatki). Te aklimatizacijske odzive bi lahko razložili z nastankom določene količine posameznega sekundarnega sporočevalca, ki lahko vpliva pri odzivnosti listnih rež na drug signal, ki je s tem sekundarnim sporočevalcem povezan. Na to hipotezo navaja več primerov iz literature (Talbot in sod., 2003; Li in sod., 2006; Lecourieux in sod., 2006)

V tem sestavku predstavljeni procesi ali zgolj orisane signalne sheme so večinoma že znanstveno potrjeni. Upoštevati moramo, da so te raziskave narejene na omejenem številu rastlinskih vrst (predvsem *Arabidopsis* in *Vicia faba*). Jasno je, da je regulacija listnih rež pri določenih rastlinskih vrstah precej drugačna (CAM, kserofiti), univerzalnost posameznih procesov v regulaciji listnih rež bo potrebno še raziskati. Vsekakor v prenosu signalov ostaja še mnogo nepojasnenih povezav, manjkajočih členov in nepoznanih medsebojnih vplivov. Mnoge elemente verig in njihove povezave je potrebno še empirično dokazati. Največji izziv pa ostaja določitev pomena posameznega člena v signalni mreži, ki regulira prevodnost listnih rež.

Zahvala

Avtorja se zahvaljujeta mag. Borisu Turku za mikrografije listnih rež.

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Metoda glavnih komponent: osnove in primer

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POVZETEK

V članku predstavljamo osnove metode glavnih komponent in njeno uporabo na enostavnem primeru. Izračuni so bili narejeni s programom SPSS.

Ključne besede: soodvisnost, metoda glavnih komponent

ABSTRACT

PRINCIPAL COMPONENT ANALYSIS: THEORY AND ILLUSTRATION

The paper presents the essential elements of the principal component analysis. We illustrate its use on a simple example. Calculations were done with the SPSS program.

Key words: interdependence, principal component analysis

1. UVOD

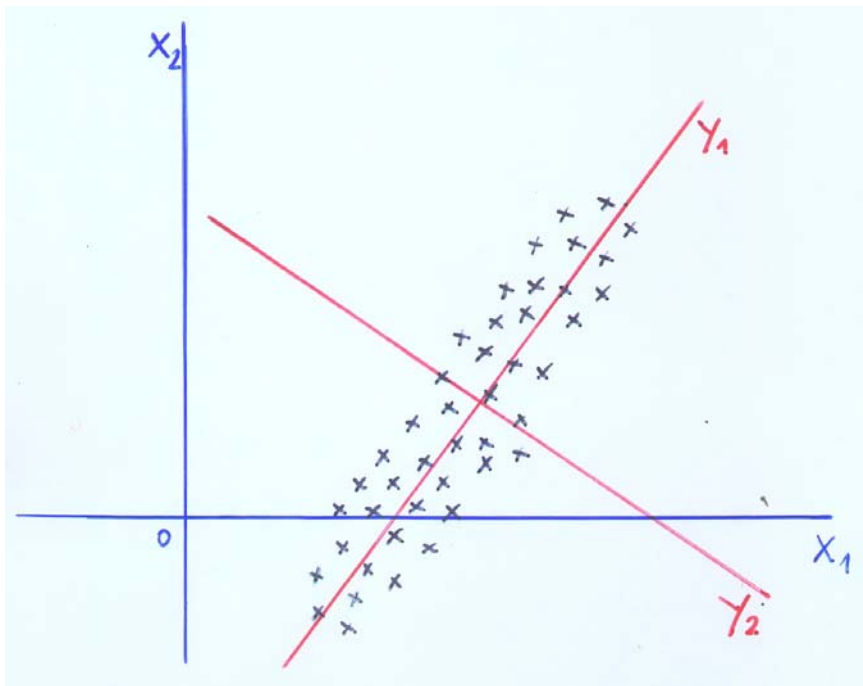
Metoda glavnih komponent (angl. *principal component analysis*, PCA) je statistična tehnika, ki analizira medsebojno soodvisnost spremenljivk z namenom, da se število spremenljivk zmanjša. Pri tem osnovni nabor spremenljivk preslikamo v množico novih spremenljivk, ki jih imenujemo *glavne komponente*.

Glavnih komponent je toliko, kolikor je osnovnih spremenljivk in so med seboj neodvisne (pravokotne). Glavne komponente se izražajo kot linearna kombinacija osnovnih spremenljivk in ohranjajo njihovo skupno variabilnost. Prva glavna komponenta je določena tako, da pojasni kar se da velik del celotne variance osnovnih spremenljivk. Druga glavna komponenta je določena tako, da je neodvisna od prve in pojasni kar se da velik del še nepojasnjene variance. Tretja glavna komponenta je neodvisna od prve in od druge glavne komponente in pojasni kar se da velik del še nepojasnjene variance, itd.

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Zaporedne glavne komponente so urejene po padajoči velikosti variance. Če so osnovne spremenljivke dovolj povezane, pojasnijo »pozne« glavne komponente majhen delež celotne variance in jih lahko zanemarimo. Bolj ko so izhodiščne spremenljivke med seboj povezane, bolj uspešna bo redukcija. Kot mero povezanosti uporabimo koeficient kovariance oz. korelacije, pri tem pa mora veljati, da je povezanost med spremenljivkami linearna.

Na Sliki 1 prikazujemo primer v dvorazsežnem prostoru izhodiščnih spremenljivk X_1 in X_2 ter pripadajoči glavni komponenti Y_1 in Y_2 . Ker je povezanost med X_1 in X_2 velika, lahko Y_1 uspešno nadomesti obe izhodiščni spremenljivki X_1 in X_2 . Dvorazsežni prostor reduciramo v enorazsežnega, pri tem pa je izguba informacije minimalna.



Slika 1. Primer v dvorazsežnem prostoru: X_1 in X_2 sta izhodiščni spremenljivki, podatki so grafično prikazani kot točke. Y_1 in Y_2 sta dobljeni glavni komponenti. Dvorazsežni prostor lahko reduciramo v enorazsežnega, ki ga določa Y_1 (slika povzeta po Ferligoj, A.).

Figure 1. X_1 and X_2 are the original variables, the data are represented by points. Y_1 and Y_2 are the corresponding principal components. Two-dimensional space can be reduced to the one-dimensional space defined by Y_1 (Figure by Ferligoj, A.).

2. MATEMATIČNO OZADJE

2.1 Ideja metode glavnih komponent

Imamo p spremenljivk X_1, X_2, \dots, X_p , ki jih zapišemo v matriko \mathbf{X} ,

$$\mathbf{X} = [X_1, X_2, \dots, X_p] \quad .$$

Naš namen je najti novo množico spremenljivk Y_1, Y_2, \dots, Y_p , ki jih zapišemo kot linearno kombinacijo osnovnih spremenljivk

$$Y_1 = a_{11}X_1 + a_{12}X_2 + \dots + a_{1p}X_p = \mathbf{X}\mathbf{a}_1$$

$$Y_2 = a_{21}X_1 + a_{22}X_2 + \dots + a_{2p}X_p = \mathbf{X}\mathbf{a}_2$$

...

$$Y_p = a_{p1}X_1 + a_{p2}X_2 + \dots + a_{pp}X_p = \mathbf{X}\mathbf{a}_p$$

Pri tem velja: $Var(Y_i) = Var(\mathbf{X}\mathbf{a}_i) = \mathbf{a}_i^T \boldsymbol{\Sigma} \mathbf{a}_i$, $Cov(Y_i, Y_k) = \mathbf{a}_i^T \boldsymbol{\Sigma} \mathbf{a}_k$,

pri čemer je $\boldsymbol{\Sigma}$ variančno-kovariančna matrika med osnovnimi spremenljivkami X_1, X_2, \dots, X_p .

Prvo glavno komponento želimo določiti tako, da je linearna kombinacija osnovnih spremenljivk: $Y_1 = \mathbf{X}\mathbf{a}_1$, ki maksimizira $Var(Y_1)$ pri dodatnem pogoju $\mathbf{a}_1^T \mathbf{a}_1 = 1$ (opomba: ta pogoj določa, da je problem enolično rešljiv). Druga glavna komponenta je linearna kombinacija $Y_2 = \mathbf{X}\mathbf{a}_2$, ki maksimizira $Var(Y_2)$ pri pogoju $\mathbf{a}_2^T \mathbf{a}_2 = 1$ in je pravokotna na prvo glavno komponento: $Cov(Y_1, Y_2) = 0$. Ta postopek nadaljujemo do zadnje, to je p -te, komponente.

Koeficiente linearnih kombinacij zapišemo v matriko \mathbf{A} . Kako dobiti koeficiente matrike \mathbf{A} ? Navajamo izrek brez dokaza.

Izrek

Glavne komponente Y_1, Y_2, \dots, Y_p se izražajo kot linearna kombinacija izhodiščnih spremenljivk: $Y_i = \mathbf{X}\mathbf{a}_i$, pri čemer velja:

- vektorji \mathbf{a}_i so lastni vektorji matrike $\boldsymbol{\Sigma}$. Rešujemo torej sistem: $|\boldsymbol{\Sigma} - \lambda \mathbf{I}| = 0$;
- varianca posamezne glavne komponente je enaka pripadajoči lastni vrednosti:
 $Var(Y_i) = Var(\mathbf{X}\mathbf{a}_i) = \mathbf{a}_i^T \boldsymbol{\Sigma} \mathbf{a}_i = \mathbf{a}_i^T \lambda \mathbf{I} \mathbf{a}_i = \lambda_i$;
- zaporedne lastne vrednosti so urejene po velikosti:
 $\lambda_1 \geq \lambda_2 \geq \dots \geq \lambda_p \geq 0$.

Posledica

Vsota varianc osnovnih spremenljivk je enaka vsoti varianc glavnih komponent, kar je $\sum_{i=1}^p \lambda_i$. Delež celotne variance, ki ga pojasnjuje i -ta glavna komponenta, je

$\lambda_i / \sum_{i=1}^p \lambda_i$. Skupni vpliv prvih m glavnih komponent, $m < p$, je $\sum_{i=1}^m \lambda_i / \sum_{i=1}^p \lambda_i$. Ta

vrednost predstavlja ključno mero uspešnosti določanja glavnih komponent.

2.2 Izračun glavnih komponent iz korelacijske matrike

Na določanje glavnih komponent najbolj vpliva tista osnovna spremenljivka, ki ima največjo varianco. Če so osnovne spremenljivke merjene v različnih merskih enotah oz. če imajo iste merske enote in različen velikostni red vrednosti, je smiselno vpliv spremenljivk izenačiti. V takih primerih osnovne spremenljivke standardiziramo in s tem dosežemo, da imajo enaka povprečja in enake variance (povprečje 0 in varianca 1). Standardizirane spremenljivke označimo Z_1, Z_2, \dots, Z_p .

V postopku določanja glavnih komponent se namesto variančno-kovariančne matrike Σ uporabi korelacijska matrika ρ . Torej gre v tem primeru za izračun lastnih vektorjev in lastnih vrednosti korelacijske matrike. Odstotek pojasnjene celotne variance s posamezno glavno komponento je $100 \cdot \frac{\lambda_i}{p}$, saj je skupna varianca v tem

primeru enaka številu spremenljivk p , skupni vpliv prvih m komponent pa je $\sum_{i=1}^m \lambda_i / p$.

Zavedati se moramo, da so rezultati, ki jih dobimo iz variančno-kovariančne matrike oz. iz korelacijske matrike, različni. Zato je treba vsakič premisliti, katera od matrik je bolj primerno izhodišče za analizo.

2.3 Reskalirani lastni vektorji

Lastne vektorje interpretiramo v smislu koeficientov pri multipli regresiji: a_{ij} predstavlja vpliv X_j na Y_i ob upoštevanju vseh ostalih osnovnih spremenljivk. Obrazložitev v smislu bivariatne analize imajo vektorji \mathbf{c}_i , ki jih dobimo tako, da lastne vektorje množimo z ustrežno konstanto: $\mathbf{c}_i = \mathbf{a}_i \sqrt{\lambda_i}$ (angl. *rescaled eigenvectors*). Za posamezni reskalirani lastni vektor \mathbf{c}_i velja, da je vsota kvadratov njegovih komponent enaka pripadajoči lastni vrednosti:

$$\mathbf{c}_i^T \mathbf{c}_i = \lambda_i \mathbf{a}_i^T \mathbf{a}_i = \lambda_i.$$

Te vektorje zložimo v matriko \mathbf{C} (angl. *component matrix*), vsak stolpec predstavlja posamezni reskalirani lastni vektor \mathbf{c}_i . Vsak element matrike \mathbf{C} predstavlja korelacijo med standardizirano osnovno spremenljivko in glavno komponento:

$$c_{ij} = \rho(Z_i, Y_j).$$

Posledica: iz vrednosti korelacijskih koeficientov v tej matriki se pogosto da najti vsebinsko obrazložitev glavnih komponent. Poudarek posvečamo velikim korelacijskim koeficientom.

3. GLAVNE KOMPONENTE NA PODATKIH

V praksi imamo vzorec velikosti n , na vsaki enoti zberemo podatke za p spremenljivk X_1, X_2, \dots, X_p . Za i -to spremenljivko podatke uredimo v vektor, $X_i = [x_{i1}, x_{i2}, \dots, x_{in}]^T$. Matrika podatkov je dimenzije $n \times p$. Iz podatkov izračunamo vzorčno variančno-kovariančno matriko \mathbf{S} oz. vzorčno korelacijsko matriko \mathbf{R} ter njene lastne vrednosti in lastne vektorje.

3.1 Ali je metoda glavnih komponent za podatke smiselna ?

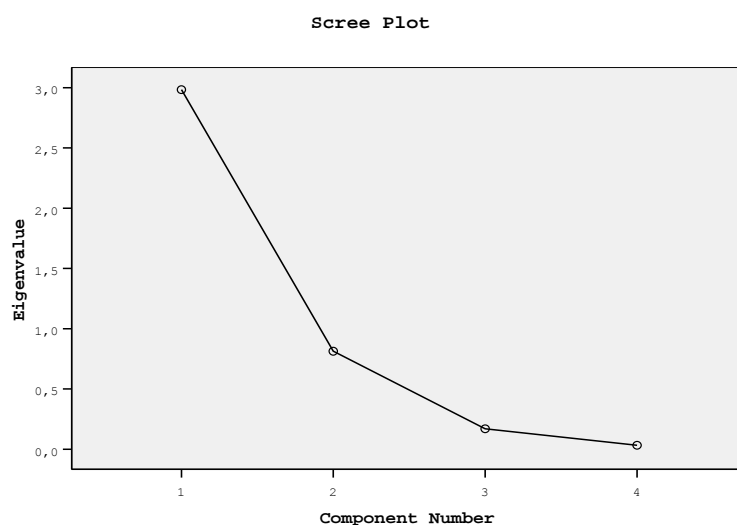
Ali je povezanost spremenljivk dovolj velika, da je smiselno nadomestiti izhodiščne spremenljivke z glavnimi komponentami? Če lahko privzamemo, da so naši podatki vzorec iz populacije ter večrazsežno normalno porazdelitev osnovnih spremenljivk, smemo uporabiti določene statistične teste. Ničelna domneva pravi, da je populacijska variančno-kovariančna matrika Σ diagonalna oz. da je populacijska korelacijska matrika ρ enaka identični matriki. Metoda glavnih komponent je smiselna, če H_0 zavrnilo pri dovolj majhnem tveganju. Najbolj znan test v te namene je Bartlettov test, ki temelji na hi-kvadrat statistiki. Dejstvo je, da je omenjena predpostavka v praksi redko izpolnjena, posledično je uporabnost tega testa relativno majhna.

Druga mera, ki služi v isti namen, je Kaiser-Meyer-Olkin-ova mera (KMO). Njena definicija temelji na vrednostih korelacijskih koeficientov in parcialnih korelacijskih koeficientov (Hutcheson, 1999) in vrednoti, ali bi spremenljivke lahko združili v skupine in posamezno skupino spremenljivk nadomestili z glavno komponento. Vrednost KMO mere je med 0 in 1, vrednosti blizu 1 kažejo, da bo redukcija uspešna, vrednosti pod 0,6 pa nakazujejo, da gre za nekoreliranost spremenljivk in neprimernost uporabe te metode.

3.2 Koliko glavnih komponent?

Ko izračunamo lastne vrednosti in lastne vektorje iz variančno-kovariančne oz. iz korelacijske matrike, se odločimo za redukcijo in privzamemo prvih m , $m < p$ glavnih komponent. Določanje števila m je do določene mere subjektivno, opiramo se na različne hevristične postopke. Navajamo nekatere:

- vnaprej določen prag: npr. izbrano število glavnih komponent naj pojasni 80 % skupne variabilnosti osnovnih spremenljivk;
- po grafu »scree plot«, ki prikazuje velikost lastne vrednosti glede na njeno zaporedno mesto. Lokacija »kolena« nakazuje število potrebnih komponent: do kolena vrednosti »strmo« padajo in pripadajoče glavne komponente upoštevamo, od kolena dalje so spremembe manjše in pripadajoče glavne komponente ne upoštevamo več. Na Sliki 2 je »koleno« pri drugi glavni komponenti, kar nakazuje, da sta dve glavni komponenti dovolj;
- Kaiser-jevo pravilo: $m = \text{Max}(j, \lambda_j \geq 1)$. Število glavnih komponent je enako številu lastnih vrednosti, katerih vrednost je vsaj ena. Za primer na Sliki 2 bi po tem pravilu zadoščala ena glavna komponenta.



Slika 2. »Scree plot« uporabljamo za določanje potrebnega števila glavnih komponent.

Figure 2. »Scree plot« is an exploratory plot used to determine the relevant number of principal components.

4. ILUSTRACIJA

Za 19 meteoroloških postaj v Sloveniji imamo podatke za 4 klimatske spremenljivke: povprečna letna maksimalna temperatura zraka ($X_1=temp_pmax$), povprečna letna minimalna temperatura zraka ($X_2=temp_pmin$), povprečna letna količina padavin ($X_3=padavine$) in povprečno število dni s snežno odejo na leto ($X_4=dni_sneg$). Podatki veljajo za 30-letno obdobje od leta 1961 do leta 1990 in so prikazani v Tabeli 1 (Košmelj, Breskvar Žaucer, 2006).

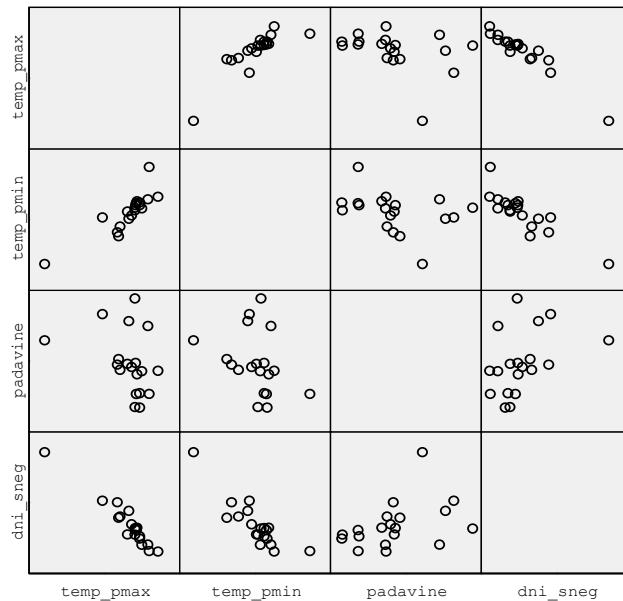
Tabela 1. Klimatski podatki za 19 meteoroloških postaj.
Table 1. Climate data for 19 meteorological stations.

Ime postaje (Name of the station)	temp_pmax (°C)	temp_pmin (°C)	padavine (mm)	dni_sneg
Babno Polje	12,1	0,1	1662	91,3
Bilje pri Novi Gorici	17,9	6,2	1456	1,8
Bizeljsko	15,2	4,9	1059	42,1
Bovec	14,5	4,5	2733	62,6
Ilirska Bistrica	15,5	4,4	1448	19,8
Kočevje	14,0	3,3	1523	73,9
Kredarica	1,2	-4,2	1993	265,1
Lendava	15,2	5,3	805	36,1
Ljubljana	14,8	5,5	1393	64,9
Maribor	14,7	5,2	1045	58,6
Murska Sobota	14,5	4,1	814	46,7
Nova vas na Blokah	12,3	1,6	1472	94,6
Portorož	16,6	10,8	1046	3,0
Postojna	13,4	3,9	1578	47,0
Rateče	11,9	0,7	1563	132,2
Stara Fužina	13,6	2,8	2333	109,7
Tolmin	16,4	5,8	2246	20,1
Vojsko	9,7	3,0	2456	136,5
Vrhnika	14,6	4,9	1594	63,9

Imamo 4 dimenzionalni prostor, v njem pa 19 enot (postaj). Ugotoviti želimo, ali lahko obravnavane 4 spremenljivke nadomestimo z manjšim številom glavnih komponent in grafično prikažemo podatke v manj dimenzionalnem prostoru. Metodo glavnih komponent uporabimo na standardiziranih spremenljivkah zaradi njihove različne narave.

Poglejmo preliminarne izpise, ki smo jih dobili s programom SPSS in so na Sliki 3 in v Tabeli 2. Kaže se močna pozitivna korelacija med *temp_pmax* in *temp_pmin* ter njuna posamična negativna korelacijo z *dni_sneg*. Spremenljivka *padavine* je statistično značilno korelirana le z *dni_sneg* ($p=0,044$), ostali dve korelaciji sta mejno statistično značilni. Točka, ki na razsevnih grafikonih »štrli ven« (nizke temperature, veliko število dni s snegom), pripada Kredarici.

Bartlettov test kaže, da ničelno domnevo, ki pravi, da je korelacijska matrika enaka identiteti, zavrnilo brez tveganja ($p=0,0000$), torej je uporaba metode glavnih komponent utemeljena. Tudi vrednost KMO mere potrjuje to trditev.



Slika 3. Matrika razsevnih grafikonov za 4 osnovne spremenljivke.

Figure 3. Scatterplot matrix for the 4 original variables.

Tabela 2. Preliminarna analiza: korelacijska matrika med obravnavanimi spremenljivkami ter testi za ugotavljanje smiselnosti uporabe metode glavnih komponent.

Table 2. Preliminary analysis: correlation matrix on the variables under study and tests for adequacy of PCA.

		temp_pmax	temp_pmin	padavine	dni_sneg
Correlation	temp_pmax	1,000	,853	-,331	-,963
	temp_pmin	,853	1,000	-,328	-,873
	padavine	-,331	-,328	1,000	,403
	dni_sneg	-,963	-,873	,403	1,000
Sig. (1-tailed)	temp_pmax		,000	,083	,000
	temp_pmin	,000		,085	,000
	padavine	,083	,085		,044
	dni_sneg	,000	,000	,044	

Kaiser-Meyer-Olkin Measure of Sampling Adequacy.	,730
Bartlett's Test of Approx. Chi-Square	68,007
Sphericity	6
Sig.	,000

Rezultati metode glavnih komponent so v Tabeli 3. Prva lastna vrednost je 2,98, kar predstavlja 75 % celotne variabilnosti, druga lastna vrednost 0,81 pojasnjuje dodatnih 20 % celotne variabilnosti, tretja lastna vrednost 0,17 pojasnjuje še nadaljnje 4 %, četrta lastna vrednost je zanemarljiva v vseh pogledih.

Tabela 3. Lastne vrednosti, odstotek pojasnjene variance ter % celotne pojasnjene variance za 4 glavne komponente.

Table 3. Eigenvalues, % of variance explained and % of total variance explained for 4 principal components.

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	2,984	74,589	74,589	2,984	74,589	74,589
2	,813	20,333	94,922	,813	20,333	94,922
3	,170	4,252	99,174	,170	4,252	99,174
4	,033	,826	100,000	,033	,826	100,000

SPSS program ne posreduje informacije o lastnih vektorjih. V Tabeli 4 prikazujemo matriko **C**, vsote kvadratov ilustrirajo teorijo. V celicah matrike so korelacijski koeficienti, krepki tisk označuje pomembne korelacijske koeficiente.

Tabela 4. Matrika **C**.

Table 4. Component matrix **C**.

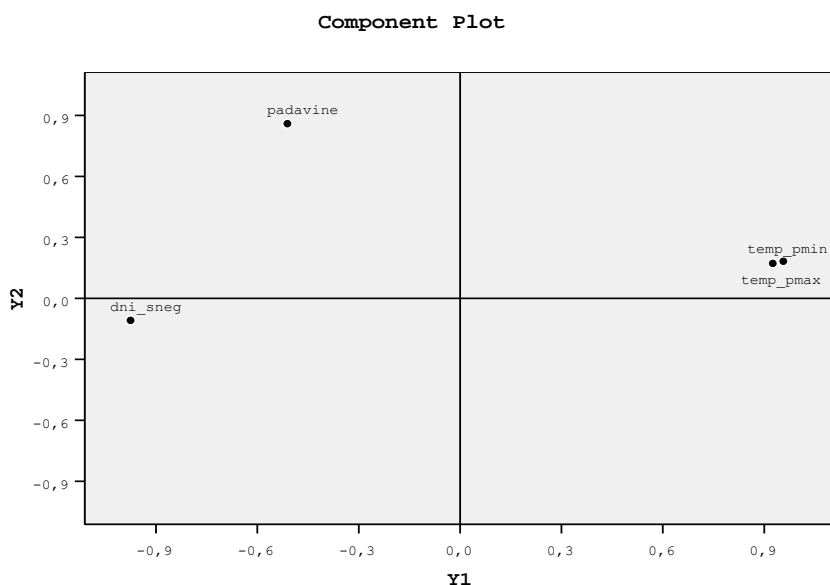
Matrika C	Y_1	Y_2	Y_3	Y_4
<i>temp_pmax</i>	0,957	0,183	-0,193	0,120
<i>temp_pmin</i>	0,925	0,172	0,340	0,013
<i>padavine</i>	-0,511	0,859	-0,001	-0,011
<i>dni_sneg</i>	-0,975	-0,108	0,137	0,136
vsota kvadratov	2,984 = λ_1	0,813 = λ_2	0,171 = λ_3	0,033 = λ_4

Sedaj se odločimo za potrebno število glavnih komponent. Slika 2 nakazuje 2 glavni komponenti, z njima bomo 4 dimenzionalni prostor zmanjšali v dvodimenzionalnega in ohranili 95 % izhodiščne informacije. Program požemo znova in zahtevamo 2 glavni komponenti. Dodatno zahtevamo, da se glavni komponenti shranita v datoteko podatkov ter sliko, ki kaže položaj originalnih standardiziranih spremenljivk v prostoru prvih dveh glavnih komponent.

Posvetimo se matriki **C** za prvi dve glavni komponenti (Tabela 4). Glede na vrednosti koeficientov korelacije med standardiziranimi osnovnimi spremenljivkami in glavnimi komponentami bomo prvo glavno komponento poimenovali »indikator temperature« (njene vrednosti so pozitivne za višje vrednosti temperature in negativne za višje število dni s snegom), drugo glavno komponento pa »padavine«. Slika 4 vizualizira položaj osnovnih standardiziranih spremenljivk v prostoru prvih dveh glavnih komponent in dopolnjuje zgornje ugotovitve.

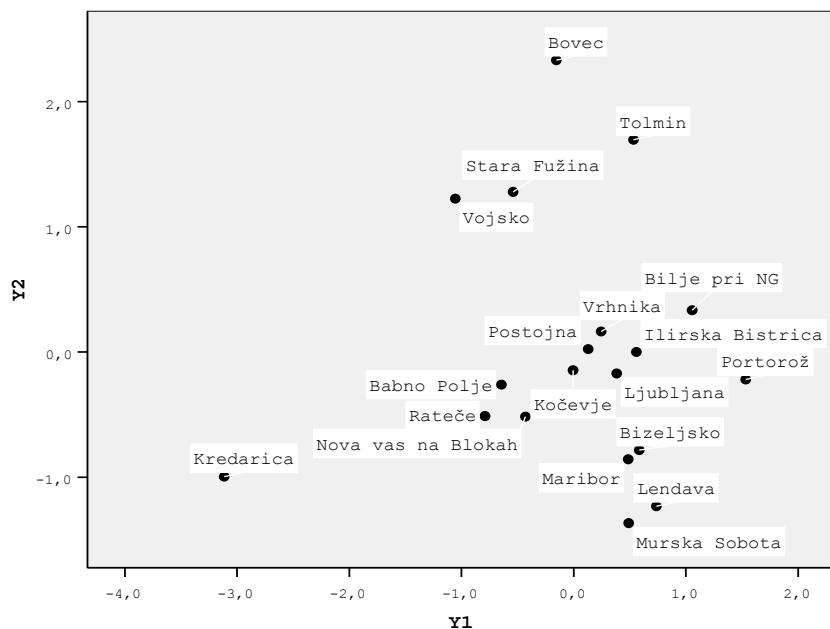
Redukcija 4-dimenzionalnega prostora v dvodimenzionalni prostor omogoča tudi grafično predstavitev postaj in interpretacijo njihove lege glede na pomen glavnih komponent. Narišimo razsevni grafikon postaj na shranjenih glavnih komponentah ter sliko dopolnimo z imeni postaj (Slika 5). Iz slike ugotovimo, da ima npr. Murska Sobota visok indikator temperature in malo padavin, Bovec srednjo vrednost

indikatorja temperature in veliko padavin, Kredarica nizke temperature in relativno malo padavin, itd.



Slika 4. Položaj osnovnih standardiziranih spremenljivk v prostoru prvih dveh glavnih komponent (SPSS: *Rotation/Display/Loadings Plot*).

Figure 4. Original standardized variables in two-dimensional space of principal components (SPSS: *Rotation/Display/Loadings Plot*).



Slika 5. Predstavitev meteoroloških postaj v dvorazsežnem prostoru glavnih komponent.

Figure 5. Representation of stations in two-dimensional space of principal components.

Analizo bomo naredili še enkrat tako, da bomo izločili postajo Kredarica, ki je potencialni osamelec. Analizirali bomo njen vpliv na rezultate. Preliminarna analiza je v Tabeli 5 in kaže, da se korelacijski koeficienti le malo spremenijo (primerjaj s Tabelo 2). Posledično se tudi rezultati metode glavnih komponent le malenkostno spremenijo (primerjaj Tabelo 4 in Tabelo 6). Slika 6 pravzaprav kaže podobno situacijo kot Slika 5, vendar bolj podrobno. Iz tega lahko sklepamo, da Kredarica glede na obravnavane spremenljivke izstopa, vendar ni osamelec.

Tabela 5. Preliminarna analiza: korelacijska matrika med obravnavanimi spremenljivkami. Izločena Kredarica.

Table 5. Preliminary analysis: correlation matrix on the variables under study. Kredarica eliminated from the analysis.

Correlation Matrix

		temp_pmax	temp_pmin	padavine	dni_sneg
Correlation	temp_pmax	1,000	,754	-,346	-,916
	temp_pmin	,754	1,000	-,284	-,771
	padavine	-,346	-,284	1,000	,423
	dni_sneg	-,916	-,771	,423	1,000
Sig. (1-tailed)	temp_pmax		,000	,080	,000
	temp_pmin	,000		,127	,000
	padavine	,080	,127		,040
	dni_sneg	,000	,000	,040	

Tabela 6. Lastne vrednosti, odstotek pojasnjene variance ter % celotne pojasnjene variance 4 in za 2 glavne komponente ter matrika C. Izločena Kredarica.

Table 6. Eigenvalues, % of variance explained and % of total variance explained for 4 and 2 principal components and component matrix C. Kredarica eliminated from the analysis.

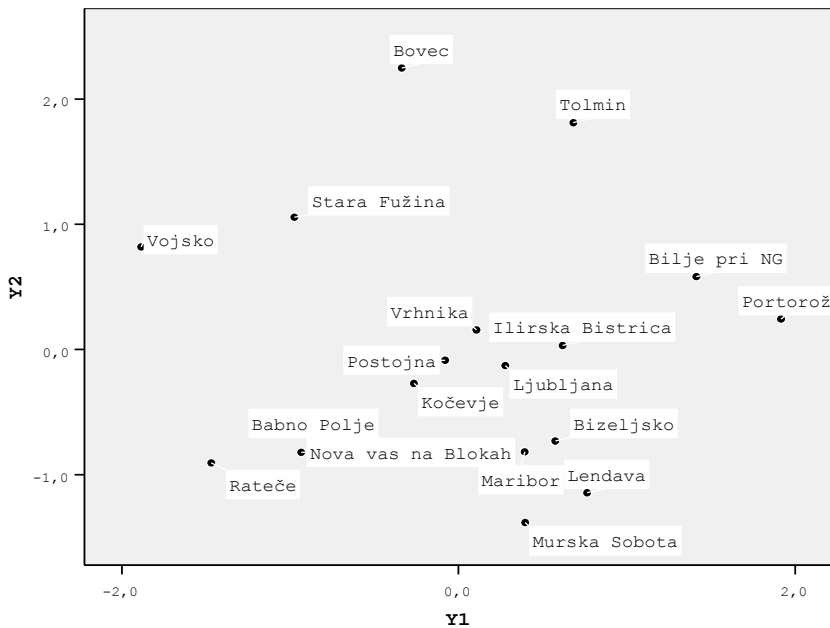
Total Variance Explained

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	2,833	70,837	70,837	2,833	70,837	70,837
2	,807	20,180	91,017	,807	20,180	91,017
3	,280	7,006	98,024			
4	,079	1,976	100,000			

Extraction Method: Principal Component Analysis.

Component Matrix

	Component	
	1	2
temp_pmax	,937	,169
temp_pmin	,870	,241
padavine	-,532	,845
dni_sneg	-,957	-,085



Slika 6. Predstavitev meteoroloških postaj v dvorazsežnem prostoru glavnih komponent. Kredarica izločena.

Figure 6. Representation of stations in the two-dimensional space of principal components. Kredarica eliminated from the analysis.

4 SKLEPI

Metoda glavnih komponent se uporablja za analizo soodvisnosti osnovnih spremenljivk s ciljem, da se zmanjša prostorska zahtevnost problema. V statističnih analizah služi predvsem za pregledovanje in raziskovanje večdimenzionalnih podatkov. Če se metoda izkaže za uspešno, dobimo globlji vpogled v izhodiščne večrazsežne podatke. Pogosto se zgodi, da je v prvih dveh/treh glavnih komponentah shranjena večina informacije (variance). V takih primerih lahko grafični prikazi v prostoru glavnih komponent razkrijejo »novosti« ter generirajo nove hipoteze o podatkih, ki jih analiziramo.

Če se redukcija izkaže kot uspešna, lahko delamo nadaljnje analize na manjšem številu glavnih komponent. To se še posebej izkaže za koristno pri določenih metodah (npr. multipla regresija, diskriminantna analiza), če:

- imamo veliko število spremenljivk za relativno majhno število enot. Če lahko osnovne spremenljivke uspešno nadomestimo z manj glavnimi komponentami, ta problem odpade;
- so osnovne spremenljivke visoko korelirane (problem multikolinearnosti). Tedaj pride do numeričnih problemov, ki se jim na ta način izognemo.

Metoda glavnih komponent nima predpostavk o verjetnostni porazdelitvi podatkov. Vendar pa izračun kovariance oz. korelacija zahteva, da so izhodiščne spremenljivke številske z vsaj ordinalno mersko lestvico. Zavedati se moramo tudi, da imajo lahko osamelci velik vpliv na izračun korelacijskih koeficientov in posledično na rezultate, zato je priporočljivo v preliminarni analizi narisati matriko razsevnih grafikonov in

narediti analizo z osamelci in brez njih. Če lahko privzamemo večrazsežno normalno porazdelitev, omogoča statistično sklepanje korak naprej.

Zahvala

Zahvaljujem se dr. Damijani Kastelec za koristne pripombe in komentarje.

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DODATEK /APPENDIX

Analizo glavnih komponent smo naredili s programom SPSS, ki ima metodo glavnih komponent vgrajeno v sklop programa **Analyze/Data Reduction/Factor**. Pri analizi rezultatov, ki jih SPSS v tem primeru posreduje, moramo biti pazljivi, kajti program je v osnovi namenjen faktorski analizi in ne metodi glavnih komponent. Posledično vsi rezultati, ki jih dobimo pri metodi glavnih komponent, niso relevantni (npr. komunalitete).

```
/* prva analiza: vse 4 glavne komponente
FACTOR
  /VARIABLES temp_pmax temp_pmin padavine dni_sneg  /MISSING LISTWISE
 /ANALYSIS temp_pmax temp_pmin padavine dni_sneg
 /PRINT UNIVARIATE INITIAL CORRELATION SIG_KMO EXTRACTION
 /PLOT EIGEN
 /CRITERIA FACTORS(4) ITERATE(25)
 /EXTRACTION PC
 /ROTATION NOROTATE
 /METHOD=CORRELATION .

/*druga analiza: zahtevamo dve glavni komponenti
FACTOR
  /VARIABLES temp_pmax temp_pmin padavine dni_sneg  /MISSING LISTWISE
 /ANALYSIS temp_pmax temp_pmin padavine dni_sneg
 /PRINT UNIVARIATE INITIAL CORRELATION SIG_KMO EXTRACTION
 /PLOT EIGEN ROTATION
 /CRITERIA FACTORS(2) ITERATE(25)
 /EXTRACTION PC
 /ROTATION NOROTATE
 /SAVE REG(ALL)
 /METHOD=CORRELATION .
```

Agrovoc descriptors: insect control; pest control; nematoda; biological control; coleoptera; scarabaeidae; curculionidae; chrysomelidae; silvanidae

Agris category codes: H10

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Entomopatogene ogorčice – biotični agensi za zatiranje žuželk iz reda Coleoptera

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IZVLEČEK

V prispevku so predstavljeni rezultati dosedanjih raziskav delovanja entomopatogenih ogorčic na gospodarsko pomembne škodljivce iz reda Coleoptera. Ti rezultati kažejo, da so lahko entomopatogene ogorčice učinkoviti biotični agensi za zatiranje hroščev iz različnih družin; Scarabaeidae, Curculionidae, Chrysomelidae in Silvanidae. S prispevkom želimo pokazati, da imajo entomopatogene ogorčice vse možnosti, da postanejo v prihodnosti tudi v Sloveniji pomemben biotični dejavnik zatiranja škodljivih hroščev, s čimer bodo lahko delno nadomestile danes prevladujočo uporabo insekticidov.

Ključne besede: entomopatogene ogorčice, hrošči, Coleoptera, Scarabaeidae, Curculionidae, Chrysomelidae, Silvanidae, biotično varstvo

ABSTRACT

ENTOMOPATHOGENIC NEMATODES – BIOLOGICAL AGENTS FOR CONTROLLING COLEOPTERAN SPECIES

The results of previous research on entomopathogenic nematodes activity against harmful Coleopteran species are presented in the paper. These results showed that the nematodes in question can be an efficient biological control agents against the Scarabaeidae, Curculionidae, Chrysomelidae and Silvanidae species. The aim of this paper is to prove the fact that also in Slovenia entomopathogenic nematodes have all possibilities to become an important biological agents as a substitute for insecticides which use in controlling harmful Coleopteran species still prevails in the country.

Key words: entomopathogenic nematodes, beetles, Coleoptera, Scarabaeidae, Curculionidae, Chrysomelidae, Silvanidae, biological control

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1 UVOD

Uporaba entomopatogenih ogorčic kot načina biotičnega varstva rastlin pred škodljivimi žuželkami je dobro znana (Kaya in Gaugler, 1993; Helyer *et al.*, 1995). Entomopatogene ogorčice živijo v sožitju z bakterijami, ki jih ogorčice po infekciji sprostijo v hemolimfni sistem gostitelja (Gaugler, 2002). Sposobnost infekcije gostitelja imajo le infektivne ličinke (IJs), ki v posebnih črevesnih veziklih prenašajo simbiotsko bakterijo (Kaya, 2000). Gostitelj, ki so ga napadle entomopatogene ogorčice, navadno umre zaradi zastrupitve ali odpovedi nekaterih organov v 24 do 72 urah po infekciji (Forst in Clarke, 2002; Smart, 1995).

Rezultati številnih raziskav so pokazali, da so entomopatogene ogorčice (v nadaljevanju uporabljamo tudi okrajšavo EPO) v visokih koncentracijah v povezavi z ugodnimi abiotičnimi dejavniki (visoka vlaga, optimalna temperatura) učinkoviti biotični agensi za zatiranje odraslih osebkov iz reda Coleoptera (Journey and Ostlie, 2000; Trdan *et al.*, 2006). Dosedanje raziskave so potrdile njihovo učinkovitost pri zatiranju odraslih osebkov japonskega hrošča (*Popillia japonica* Newman) (Lacey *et al.*, 1993; Grewal *et al.*, 2002; Koppenhöfer in Fuzy, 2007), koruznega hrošča (*Diabrotica virgifera virgifera* LeConte) (van der Burgt *et al.*, 1998; Toepfer, 2005) hrošča *Typhaea stercorea* (L.) (Svendsen and Steenberg, 2000), črnega žitnega žužka (*Sitophilus granarius* [L.]) (Trdan *et al.*, 2006), surinamskega mokaarja (*Oryzaephilus surinamensis* [L.]) (Trdan *et al.*, 2006), kapusove bolhače (*Phyllotreta* spp.) (Laznik, 2006) in še nekaterih drugih predstavnikov iz reda Coleoptera.

Številne druge raziskave so pokazale, da so EPO pri manjših koncentracijah mnogo bolj učinkovite pri zatiranju preimaginalnih stadijev žuželk iz reda Coleoptera (Theunis, 1998; Converse in Grewal, 1998; Ansari *et al.*, 2003). Podobne ugotovitve so mnogi potrdili tudi pri zatiranju žuželk iz drugih redov; Thysanoptera (Ebbsa, 2005; Premachandra *et al.*, 2003a; Helyer *et al.*, 1995), Lepidoptera (Shamseldeen in Ismail, 1997; Yakir *et al.*, 1998), Diptera (Nielsen in Philipson, 2004; Hara *et al.*, 1993) in še nekaterih drugih. V večini zgledov je šlo za ličinke, ki pretežni del svojega razvoja preživijo v tleh in so zaradi tega mnogo bolj ustrezne za preučevanje učinkovitosti EPO, ki so talni organizmi (Gaugler, 2002).

2 PREGLED DOSEDANJIH OBJAV

2.1 Družina Scarabaeidae

Ogreci so ličinke hroščev iz družine skarabejev (Scarabaeidae), ki se prehranjujejo s podzemnimi deli rastlin in s tem velikokrat povzročajo precejšnjo škodo; ta je navadno največja na travni ruši. Med vsemi hrošči je bilo delovanje EPO doslej najbolj intenzivno preučevano prav na predstavnikih te družine (Klein, 1990, 1993). Ker so ogreci talni škodljivci, je bilo v preteklih letih iz mrtvih osebkov izoliranih več vrst EPO (Peters, 1996). V evoluciji so omenjeni škodljivci razvili številne obrambne mehanizme, ki vplivajo na različno stopnjo njihove odpornosti na različne vrste EPO (Gaugler, 2002).

Sploh prva izolacija EPO je bila leta 1923 iz hrošča *Popillia japonica*, omenjenega škodljivca pa so v prvem poljskem poskusu z EPO zatirali že v štiridesetih letih prejšnjega stoletja (Glaser, 1932; Fleming, 1968). Klein (1990, 1993) je v tej zvezi poročal o boljši učinkovitosti vrste *Steinernema glaseri* v primerjavi z vrsto *S. feltiae*. Georgis in Gaugler (1991) sta ugotovila, da je učinkovitost vrste *Heterorhabditis bacteriophora* za zatiranje vrste *P. japonica* ob ustreznem nanosu primerljiva z insekticidi. Grewal *et al.* (2002) poročajo, da sta vrsti *H. zealandica* sev X1 in *H. bacteriophora* sev GPS11 povzročili več kot 50% smrtnost žuželke *P. japonica*, medtem ko sta bili vrsti *H. indica* in *H. marelatus* manj učinkoviti, saj sta povzročili okrog 20% smrtnost osebkov.

Na učinkovitost EPO pri zatiranju ogrcev vplivajo interakcije med različnimi biotičnimi in abiotičnimi dejavniki. Med slednjimi sta pomembna vsebnost organske snovi v tleh - ta zavira premikanje ogorčic v nižje talne plasti (Georgis and Gaugler, 1991; Zimmerman and Cranshaw, 1991) - in stopnja vlažnosti tal (Shetlar *et al.*, 1988; Georgis in Gaugler, 1991). Ogorčice so bolj učinkovite v fino strukturiranih tleh, ker je njihova mobilnost v višje plasti boljša, tam pa se zadržuje več ogrcev (Georgis in Gaugler, 1991). Znano je, da so precej bolj učinkovite za zatiranje ogrcev tiste vrste EPO, ki iščejo gostitelje aktivno. Med te štejemo vrste iz rodu *Heterorhabditis* in vrsto *S. glaseri*. Ogorčice, ki čakajo na žrtve v zasedi (na primer vrsta *S. carpocapsae*) so navadno manj učinkovite (Gaugler *et al.*, 1997a). Prav to dejstvo pojasnjuje rezultate predhodnih raziskav (Georgis in Gaugler, 1991; Georgis in Poinar, 1994), ko je bila ugotovljena različna stopnja učinkovitosti različnih vrst EPO.

Najboljši odgovor na enkapsulacijo, način obrambnega mehanizma, ki je značilen tudi za vrsto *P. japonica*, ima vrsta *S. glaseri* (Cui *et al.*, 1993; Wang *et al.*, 1995). Ta vrsta obrambe je pri vrsti *H. bacteriophora* povzročila kar 75% smrtnost, medtem ko je bila ta pri vrsti *S. glaseri* le 12%. V sorodni raziskavi so ugotovili, da je vrsta *S. glaseri* najbolj učinkovita (76,5%) za zatiranje hrošča *Cyclocephala hirta* LeConte (Converse in Grewal, 1998), medtem ko so bile manj učinkovite ogorčice *S. feltiae*, *S. anomali*, *S. kushidai*, *H. bacteriophora* in *H. megidis*. Vrsti *S. carpocapsae* in *S. riobravus* nista pokazali patogenosti za omenjenega škodljivca travne ruše. Zato neuspešnost nekaterih poskusov, ko se določene vrste EPO niso izkazale za učinkovite agense pri zatiranju hroščev iz družine Scarabaeidae, lahko pripisujemo napačni izbiri vrst ogorčic (Converse in Grewal, 1998). Ogorčice, še posebno vrsta *H. bacteriophora* postanejo manj infektivne za ogrce, ko pade temperatura tal pod 20°C (Georgis in Gaugler, 1991).

Hrošč *Anomala orientalis* Waterhouse je manj občutljiv na delovanje vrste *H. bacteriophora* kot hrošč *P. japonica*, medtem ko sta vrsti *Rhizotrogus majalis* (Razoumowsky) in *Maladera castanea* (Arrow) popolnoma odporni na napad omenjene ogorčice (Koppenhöfer *et al.*, 2006). Vrsta *S. scarabaei*, ki so jo izolirali iz ogrcev v New Jersey-u, je na prostem najučinkoviteje delovala na vrste *P. japonica*, *A. orientalis* in *R. majalis*. Omenjena ogorčica je pokazala izjemen potencial v biotičnem zatiranju ogrcev. Vse ostale vrste ogorčic v poskusu (*H. bacteriophora*, *H. zealandica* in *S. glaseri*) so bile prav tako učinkovite, a predvsem v laboratorijskih razmerah (Koppenhöfer and Fuzy, 2007).

Različne stopnje ličink omenjenih škodljivcev kažejo razlike v občutljivosti na napad EPO (Deseö *et al.*, 1990; Fujiie *et al.*, 1993; Smits *et al.*, 1994). Za hrošča *Rhizotrogus majalis* so ugotovili, da je v drugi larvalni stopnji mnogo bolj občutljiv na delovanje entomopatogene ogorčice *H. bacteriophora* kot v tretji larvalni stopnji. Osebkii tretje larvalne stopnje so namreč večji in za njihovo zatrtje je potrebna višja koncentracija suspenzije ogorčic (Taesdale in Henderson, 2003). Do podobnih ugotovitev so prišli tudi Simard *et al.* (2001), ki so obenem ugotovili tudi, da je učinkovitost ogorčic večja v peščenih tleh.

Costello (2003) je v laboratorijskih razmerah primerjal učinkovitost nekaterih insekticidov in EPO za zatiranje ličink hrošča *Rhizotrogus majalis*. Vrsti *S. feltiae* in *H. bacteriophora* sta bili zelo učinkoviti (71%), njuno delovanje pa je bilo primerljivo z insekticidi, ki se uporabljajo za zatiranje omenjenih žuželk v konvencionalnem kmetijstvu.

Entomopatogeni ogorčici *S. glaseri* in *H. megidis* sta bili bolj učinkoviti kot vrsta *S. feltiae* za zatiranje tretjega larvalnega stadija skarabeja *Hoplia philanthus* Füssly. Izračunani LC₅₀ in LC₉₀ vrednosti sedmi dan po tretiranju sta bili za najbolj učinkoviti vrsti ogorčic 4,6 oz. 9,7 ter 79,3 oz. 511,8 IJs/ogrca. Omenjene raziskovalce je zanimalo, če je učinkovitost ogorčic za zatiranje tega škodljivca večja ob sočasnem nanosu entomopatogene glive *Metarhizium anisopliae* (Metschnikoff) Sorokin. Pri koncentraciji suspenzije 5×10^{12} konidijev/ha in $2,5 \times 10^9$ IJs/ha (*H. bacteriophora*) je bila ugotovljena kar 95% stopnja smrtnosti škodljivca. Kombinacija glive in EPO se je pokazala za učinkovit način zatiranja hrošča *H. philanthus* in se priporoča v integriranem varstvu rastlin, nadaljnje raziskave pa bodo pokazale ali izbrana kombinacija učinkovito deluje tudi na druge vrste škodljivih žuželk (Ansari *et al.*, 2006).

V laboratorijskem poskusu (Theunis, 1998) so preučevali učinkovitost šestih vrst EPO - *S. glaseri* (#326 in nc 34, gb), *S. feltiae*, *S. carpocapsae* in *H. zealandica* - za zatiranje hrošča *Papuana uninodis* Prell. Najboljše rezultate je pokazala vrsta *S. glaseri* (#326), za katero je bila pri omenjenem škodljivcu LD₅₀ vrednost 7000 IJs/ogrca (en dan po nanosu) oz. 250 IJs/ogrca (6 dni po nanosu). Nadaljnji poskusi ugotavljanja učinkovitosti ogorčic na istega škodljivca na prostem niso dali zadovoljivih rezultatov.

Za vrsto *Popillia japonica* je bilo ugotovljeno, da odgovori na napad entomopatogenih ogorčic na prav poseben način. Z drgnjenjem nog ob telo namreč iz površja telesa odstranjuje ogorčice. Na ta način naj bi žuželka odstranila 60% napadalcev, vendar pa so ostale ogorčice vseeno sposobne povzročiti smrt žuželke, a v znatno nižjem odstotku (~25%) (Gaugler *et al.*, 1994).

2.2 Družina Curculionidae

Entomopatogene ogorčice iz rodov *Steinernema* in *Heterorhabditis* so uspešni biotični agensi tudi za zatiranje različnih vrst hroščev iz družine Curculionidae. Dosedanje raziskave govorijo o njihovem uspešnem delovanju na vrste iz rodu *Otiorynchus* (Wilson *et al.*, 1999), *Diaprepes abbreviatus* (L.) (McCoy *et al.*, 2002;

Shapiro in McCoy, 2000), *Pachnaeus litus* (Germar) (Lacey in Shapiro, 2003) in na nekatere druge (Trdan *et al.*, 2005, 2006).

Jansson in Lecrone (1994) poročata o učinkovitem delovanju EPO na rilčkarja *Cylas formicarius elegantulus* (Summers). Ogorčice iz rodu *Heterorhabditis* so se izkazale za bolj učinkovite od ogorčic iz rodu *Steinernema* (Jansson *et al.*, 1990, 1993).

V laboratorijskih razmerah je bila preučevana tudi učinkovitost zatiranja različnih vrst EPO v odvisnosti od koncentracije suspenzije in temperature na hroščke črnega žitnega žužka (*Sitophilus granarius*). Rezultati raziskave so pokazali, da koncentracija suspenzije ni vplivala na smrtnost obravnavanega škodljivca, medtem ko sta se vrsta ogorčic in temperatura izkazala za signifikantna dejavnika učinkovitosti ogorčic. Smrtnost hroščkov je bila največja pri 20 in 25°C, v poskusu, ki je vključeval štiri vrste ogorčic - *S. feltiae*, *S. carpocapsae*, *H. bacteriophora* in *H. megidis* -, pa se je le slednja izkazala kot neučinkovita. Najboljše delovanje je pokazala vrsta *H. bacteriophora* pri 25°C, ko je povzročila 81% smrtnosti hroščkov (Trdan *et al.*, 2005, 2006).

Entomopatogena ogorčica *S. carpocapsae* je bila prva, za katero je bilo dokazano učinkovito delovanje na rilčkarja *Diaprepes abbreviatus*, ki spada med pomembnejše škodljivce citrusov (Laumond *et al.*, 1979). Uporaba ogorčice za zatiranje omenjenega škodljivca se je pozneje razširila tudi v tržni pridelavi citrusov (Smits, 1994). Številni laboratorijski poskusi in poskusi v rastlinjakih so pokazali večjo učinkovitost omenjenega agensa v primerjavi z vrstami *S. glaseri*, *S. feltiae* in *H. bacteriophora* v biotičnem zatiranju *D. abbreviatus* (Schroeder, 1987; Figueroa in Roman, 1990). Vendar pa je bila učinkovitost ogorčice v laboratorijskih poskusih boljša kot se je pozneje pokazalo na prostem, kjer je bila manjša od 70% (Gaugler, 2002). Poznejše laboratorijske in poljske raziskave so pokazale, da je za zatiranje rilčkarja bolj učinkovita vrsta *S. riobravo* (Schroeder, 1994; Duncan *et al.*, 1996; Bullock *et al.*, 1999b). Stuart *et al.* (2004) poročajo, da se je učinkovitost vrste *S. riobravo* pri zatiranju rilčkarja *D. abbreviatus* gibala od 57 do 84%. Omenjena raziskava je potrdila rezultate predhodnjih (Duncan in McCoy, 1996; Shapiro *et al.*, 1999; Shapiro in McCoy, 2000). V številnih raziskavah je omenjena vrsta ogorčic povzročila tudi več kot 90% smrtnost ličink omenjenega škodljivca (Duncan in McCoy, 1996; Duncan *et al.*, 1996; Bullock *et al.*, 1999b).

Trenutno se za zatiranje vrste *D. abbreviatus* tržijo le pripravki na podlagi vrst *S. riobrave* in *H. indica* (Lacey in Shapiro, 2003). McCoy *et al.* (2002) so preučevali delovanje pripravka GrubstakeTM 100, katerega aktivno snov predstavljajo infektivne ličinke vrste *H. indica* ter Bio Vector 355, v katerem so aktivna snov infektivne ličinke vrste *S. riobrave*. Dobljeni rezultati niso bili skladni s pričakovanji, saj je bila učinkovitost obeh pripravkov precej nizka. Razlago lahko iščemo v dejstvu, da na učinkovitost EPO vpliva več dejavnikov (vrsta gostitelja, okolje, stopnja ličinke, temperatura tal) (Kaya in Gaugler, 1993), koncentracija ogorčic v suspenziji ali v tleh (Shapiro-Ilan *et al.*, 2002; McCoy *et al.*, 2000), formulacija pripravka (Shapiro-Ilan in McCoy, 2000) in tip tal (Shapiro-Ilan *et al.*, 2000; McCoy *et al.*, 2002).

Rilčkar *Otiorynchus sulcatus* (Fabricius) spada med gospodarsko najpomembnejše škodljivce brusnic, jagod in okrasnih rastlin v Severni Ameriki in Zahodni Evropi. V

Severni Ameriki za zatiranje omenjenega škodljivca na leto porabijo od 25 do 70 milijonov dolarjev (Shapiro-Ilan *et al.*, 2006), na Nizozemskem pa od 500.000 do 2 milijona dolarjev (van Tol in Raupp, 2006). Poljski poskusi so pokazali, da so ogorčice iz rodu *Heterorhabditis* bolj učinkovite od tistih iz rodu *Steinernema* (van Tol in Raupp, 2006; van Tol *et al.*, 2004). Najpomembnejši omejujoči dejavnik pri zatiranju tega rilčkarja z EPO je temperatura tal, optimalen čas za uporabo ogorčic pa je pozno spomladi (konec aprila, maj) (van Tol *et al.*, 2004). V sorodni raziskavi so Wilson *et al.* (1999) preučevali učinkovitost dveh vrst EPO (*H. bacteriophora* in *H. marelatus*) za zatiranje vrste *O. sulcatus*. Obe ogorčici sta pomembno zmanjšali število ličink omenjenega škodljivca. V drugi raziskavi je bilo ugotovljeno, da je vrsta *H. heliothidis* pri visokih koncentracijah (od 48000 do 80000 IJs/rastlino) uspešen biotični agens za zatiranje rilčkarja *O. sulcatus* (Curran in Patel, 1988).

Pomemben škodljivec sadnega drevja je tudi rilčkar *Curculio caryae* (Horn). Z nanosom suspenzije ogorčice *S. carpocapsae* je bila v eni od raziskav dosežena kar 80% smrtnost ličink tega škodljivca (Shapiro-Ilan *et al.*, 2003). V nekoliko starejši raziskavi je bilo ugotovljeno, da se s starostjo rilčkarjevih ličink zmanjšuje učinkovitost EPO (Shapiro-Ilan, 2001). V sorodni raziskavi (Shapiro-Ilan *et al.*, 2003) pa so potrdili, da so vse rase ogorčice *S. carpocapsae* bolj virulentne za odrasle osebe kot za ličinke. V poskus so bile vključene rase Breton, DD136, Italian in Kapow, ki so se izkazale za bolj učinkovite od ras Agriotos, All in Sal. Shapiro-Ilan *et al.* (2004) so preučevali tudi morebitni sinergizem med EPO in entomopatogenimi glivami, vendar pa rezultati sočasnega nanosa obeh biotičnih agensov niso bili bistveno boljši od rezultatov samostojnega nanosa ogorčic.

Hrošček *Cylas formicarius elegantulus* (Summers) velja za enega od najpomembnejših škodljivcev sladkega krompirja. V zadnjih dvajsetih letih intenzivno preučujejo učinkovitost različnih predstavnikov obeh rodov EPO za zatiranje tega škodljivca (Georgis *et al.*, 2006). Poljski poskusi so pokazali, da so ogorčice v tej zvezi zelo učinkovite, a predvsem pri zatiranju ličink in bub (Jansson *et al.*, 1990, 1991, 1993). Jansson *et al.* (1991) poroča o boljši učinkovitosti enkratnega nanosa ogorčic v primerjavi z večkratnim, a le če je čas, ko uporabimo ogorčice, optimalen. Koncentracija suspenzije ogorčic je bila v omenjenem poskusu zelo visoka (od 6,4 do 18,8 milijonov IJs/ha). V sorodni raziskavi je bilo ugotovljeno, da so vrste iz rodu *Heterorhabditis* (*H. bacteriophora* [HP88]) bolj učinkovite od tistih iz rodu *Steinernema*, njihovo delovanje pa je bilo v nekaterih zgledih boljše od delovanja insekticidov (Jansson *et al.*, 1993). Ekanayake *et al.* (2001) poročajo, da imajo nekatere rase ogorčice *H. megidis* (Poinar, Jackson in Klein) podobno učinkovitost pri zatiranju vrste *Cylas formicarius elegantulus*, vendar pa še vedno velja, da je cena takšnih biopripravkov omejujoč dejavnik njihove širše uporabe.

Vrsta *Listronotus oregonensis* LeConte je predvsem v Severni Ameriki znan škodljivec korenja, zelene in peteršilja (Georgis *et al.*, 2006). Optimalen čas za zatiranje škodljivca s talnim nanosom EPO nastopi spomladi, ko zapušča zimska bivališča in se seli na njive s korenjem. Miklasiewicz *et al.* (2002) poročajo, da s takšnim zgodnjim nanosom suspenzije ogorčice *H. bacteriophora* vplivamo na večjo smrtnost tega rilčkarja.

Pomemben škodljivec sladkorne pese, zlasti v zahodnem Sredozemlju (Francija, Italija, Španija), je tudi vrsta *Temnorhinus mendicus* (Gyllenhal). Škodljivec, ki ima le en rod na leto, prezimi v tleh v razvojnem stadiju odraslega osebka (Georgis *et al.*, 2006). Ugotovljeno je bilo, da so rilčkarjeve ličinke in bube občutljive na napad EPO. V poljskem poskusu so s koncentracijo 25 IJs/cm² dosegli od 90 do 95% učinkovitost ogorčic, odstotek smrtnosti rilčkarjevih ličink pa je bil večji od tistega, kjer so za njihovo zatiranje uporabili insekticide (Curto *et al.*, 1999).

2.3 Družina Chrysomelidae

Koloradski hrošč (*Leptinotarsa decemlineata* [Say]) velja za najpomembnejšega škodljivca krompirja. Številne raziskave so se osredotočale na delovanje različnih vrst ogorčic zoper četrti larvalni stadij škodljivca (Wright *et al.*, 1987). Pri foliarnem nanosu suspenzije ogorčic so zaradi hitre izsušitve ugotovili njihovo manjšo učinkovitost, čeprav so z dodatki za preprečevanje izsuševanja uspeli nekoliko podaljšati delovanje vrste *S. carpocapsae* (MacVean *et al.*, 1982). Pri simulaciji poljskega poskusa v rastnih komorah je bila učinkovitost ogorčic manjša od tiste v laboratorijskih razmerah (Stewart *et al.*, 1998; Wright *et al.*, 1987). Ogorčica *S. carpocapsae* je pri koncentraciji suspenzije 93 IJs/cm² talnega površja vplivala na 79% smrtnost četrte stopnje ličink koloradskega hrošča, medtem ko je bila vrsta *H. bacteriophora* pri višji koncentraciji (155 IJs/cm² talnega površja) manj učinkovita (67%). Poskusi v rastlinjaku, kjer so ugotavljali delovanje ogorčice *S. carpocapsae* na škodljivčeve predbube, so ugotovili boljše rezultate, saj je bila vrsta pri koncentraciji suspenzije 84 IJs/cm² talnega površja učinkovita kar 94% (Nickle *et al.*, 1994).

Berry *et al.* (1997) poročajo, da je vrsta *H. marelatus* pri nizki koncentraciji suspenzije (50 IJs/ cm² talnega površja) zelo učinkovita za zatiranje koloradskega hrošča. Do podobnih rezultatov so prišli tudi Armer *et al.* (2004a), ki so poročali, da je isti agens pri koncentraciji 50 IJs/cm² talnega površja zmanjšal populacijo odraslih osebkov za 50 %. Omenjeni raziskovalci ugotavljajo, da se ogorčice v tleh niso razmnoževale. Učinkovitost EPO na koloradskega hrošča je odvisna od številnih dejavnikov, med njimi sta pomembna globina zabubljenja (od 1 do 15 cm) in migracija hroščev iz sosednjih rastlin in njiv (MacVean *et al.*, 1982). Armer *et al.* (2004b) še ugotavljajo, da se ogorčica *H. marelatus* ter njena simbiotska bakterija *Photorhabdus luminescens* ne razmnožujeta v ličinkah in predbubah škodljivca zaradi inhibitornih snovi v njegovi hemolimfi, kar zmanjšuje njun biotični potencial.

Rezultati ločenih raziskav učinkovitosti EPO na koruznega hrošča (*Diabrotica virgifera virgifera* LeConte) se zelo razlikujejo. V eni od takšnih je bila ogorčica *S. carpocapsae* najbolj učinkovita pri zatiranju druge in tretje larvalne stopnje škodljivca (Jackson in Brooks, 1995). Učinkovitost te ogorčice v laboratorijskih razmerah je bila večja od 90% (Nickle *et al.*, 1994). O podobnih rezultatih z isto vrsto ogorčice poročajo tudi Wright *et al.* (1993). V poljskih poskusih, v katerih so zatirali odrasle osebkove koruznega hrošča, so z nanosom koncentracije suspenzije ogorčice *S. carpocapsae* (od 100.000 do 200.000 IJs/15 cm² talnega površja) dosegli zmanjšanje poškodb pod gospodarski prag škodljivosti (Jackson in Hesler, 1996). Uporaba ogorčice *S. carpocapsae* je upravičena, kadar na prostem prevladujeta druga in tretja larvalna stopnja škodljivca (Jackson, 1996). Ogorčica *S. carpocapsae* v kombinaciji z vrsto *H. bacteriophora* je v eni od raziskav vplivala na znatno zmanjšanje populacije

koruznega hrošča in ni vplivala na večjo smrtnost neciljnih organizmov (Georgis *et al.*, 1991). Sorodna raziskava je pokazala tudi, da lahko ista kombinacija ogorčic vpliva na zmanjšanje obsega poškodb pod gospodarski prag škodljivosti (Jackson, 1996), pri čemer se je učinkovitost ogorčic gibala od 66 do 98%. Pri visokih koncentracijah suspenzije vrste *S. carpocapsae* je bilo doseženo boljše delovanje na drugo larvalno stopnjo koruznega hrošča (Journey in Ostlie, 2000). Toepfer *et al.* (2005) poročajo, da so ogorčice *S. glaseri*, *S. arenarium*, *S. abassi* in *H. bacteriophora* v koncentracijah od 7,9 do 15,9 IJs/cm² talnega površja povzročile prek 77% smrtnost škodljivca v tretji larvalni stopnji.

Čeprav so kapusovi bolhači (*Phyllotreta* spp.) eni od pomembnejših škodljivcev kapusnic, tako v Evropi kot na nekaterih drugih celinah, doslej za njihovo zatiranje še niso uporabljali EPO. Edini doslej objavljeni vir so rezultati raziskave, ki smo jo v letu 2005 izvajali na Katedri za entomologijo in fitopatologijo Oddelka za agronomijo Biotehniške fakultete v Ljubljani. V laboratorijskih razmerah smo namreč preizkušali delovanje vrst *S. feltiae*, *S. carpocapsae*, *H. megidis* in *H. bacteriophora* na odrasle osebkke kapusovih bolhačev. Poskus smo izvajali pri treh različnih temperaturah (15, 20 in 25°C) in treh različnih koncentracijah suspenzije ogorčic (200, 1000 in 2000 infektivnih ličink/hrošča). Rezultati so potrdili že prej znana dejstva, da so lahko EPO v visokih koncentracijah v povezavi z ugodnimi abiotičnimi dejavniki (visoka vlaga, optimalna temperatura) učinkovit biotični agens tudi za zatiranje odraslih osebkov iz reda Coleoptera (Lacey *et al.*, 1993). Ugotovili smo še, da je aktivnost ogorčic v večji meri odvisna od temperature kot od koncentracije (Laznik, 2006). Vse štiri vrste ogorčic so bile najbolj učinkovite pri 25°C. Samo vrsta *S. feltiae* je dosegla zadovoljivo učinkovitost tudi pri najnižji temperaturi, kar je iz praktičnega vidika (nočno tretiranje) (Akalach in Wright, 1995) zagotovo prednost.

2.4 Družina Silvanidae

Štiri vrste entomopatogenih ogorčic (*S. feltiae*, *S. carpocapsae*, *H. bacteriophora* in *H. megidis*) so bile vključene v laboratorijski poskus zatiranja skladiščnega škodljivca *Oryzaephilus surinamensis*. Učinkovitost ogorčic so raziskovalci ugotavljali pri treh različnih temperaturah (15, 20 in 25°C) ter treh različnih koncentracijah suspenzije ogorčic (500, 1000 in 2000 IJs/odrasli osebek). Najslabše delovanje na škodljivca je pokazala vrsta *H. megidis*, medtem ko med ostalimi vrstami ogorčic ni bilo statistično značilnih razlik v delovanju. Najboljša učinkovitost agensov je bila ugotovljena pri 20°C (LC₅₀ 921-1335 IJs/odrasli osebek). Koncentracija suspenzije ogorčic se je v poskusu izkazala kot manj pomemben dejavnik učinkovitosti ogorčic (Trdan *et al.*, 2006).

3 ZAKLJUČKI

Entomopatogene ogorčice so v svetu že dobri znani biotični agensi za zatiranje škodljivih žuželk, v Sloveniji pa pričakujemo njihovo širšo uporabo že v bližnji prihodnosti, ko bodo izgubile status tujerodnih organizmov. V tem prispevku predstavljeni rezultati tujih in domačih laboratorijskih raziskav kažejo na precejšen biotični potencial EPO pri zatiranju škodljivih hroščev, ki so tudi pri nas ena od pomembnejših žuželčjih skupin, ki zmanjšuje kakovost in količino gojenih in

samoniklih rastlin. Zato pričakujemo, da bo imela ta skupina biotičnih agensov v Sloveniji v prihodnosti precejšen pomen pri zatiranju gospodarsko škodljivih hroščev.

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Die Wälder der Herrschaften Žiče und Frajštanj und ihre Bewirtschaftung im Übergang vom 18. ins 19. Jahrhundert

Jože MAČEK¹

ABSTRACT

THE FORESTS OF THE ESTATES ŽIČE AND FRAJŠTANJ AND THEIR ECONOMY IN THE PERIOD BETWEEN THE END OF THE EIGHTEENTH AND THE BEGINNING OF THE NINETEENTH CENTURY

The subject of the treatise are the forests (woods) and their economy on Žiče and Frajštanj estates in the Lower Styria, which were the property of the Styrian Religion Fund, in the period between the end of the eighteenth and the beginning of the nineteenth century. The first estate possessed 2365 and the second 1423 yokes of forests. The distribution of the tree species and the state of the forest stands, which were with some exceptions generally bad, are described. Detailed use of the forests, that were shared on one side between the bondmen, in the form of servitude rights (rights for felling the trees for firewood and building material, rights for the pasture of livestock and swines separately, and the rights to rake up the strewing) and on the other side the rights of the estate owners, are discussed. In that time the forests were to a great extent included in the husbandry of the bondmen farms. The estates did not have the power to reduce the servitude rights, which were in force for ages. To small extent they attempted to regulate them, but with little success. The servitude rights were a great obstacle for good administration of the forests. The estate Žiče tried to solve this problem through the cession (separation) of their forests parts to the bondmen, which should denounce to their servitude rights on the remaining estate forests. But during the period in question the intention was not realized. These problems are extensively discussed in archival sources. On the estate Frajštanj the cession (separation) of the forests to bondmen, for their denouncing to the servitude rights, has not been a topic yet. On both estates there no traces can be observed about contemporary forest managing and the incomes from their forests were negligible.

IZVLEČEK

GOZDOVI GOSPOSTEV ŽIČE IN FRAJŠTANJ IN GOSPODARJENJE Z NJIMI NA PREHODU IZ 18. V 19. STOLETJE

V razpravi so prikazani gozdovi in gospodarjenje z njimi na gospostvih Štajerskega verskega sklada Žiče in Frajštanj na prehodu iz 18. v 19. stoletje. Prvo je imelo 2365, drugo pa 1423 oralov gozdov. Prikazana je njihova obraščenost z drevesnimi vrstami in stanje sestojev, ki je bilo razen izjem sorazmerno slabo. Obširno je obdelana njihova izraba, ki so si jo delili podložniki v obliki služnostnih pravic (pravica do drv, stavbnega in drugega lesa, paše živine, posebej še svinj ter pravice do stelje) ter seveda gospostvo. Gozdovi so bili tedaj še v velikem obsegu vključeni v gospodarjenje podložniških kmetij. Služnostne pravice so izvirale iz davnih časov in se jih gospostvi načeloma nista upali dotikati, poskušali sta jih samo nekoliko (z malo uspeha) uravnavati. Ker so bile služnostne pravice izjemno velika ovira za sodobno

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gospodarjenje z gozdovi, se jih je vsaj gospostvo Žiče skušalo rešiti tako, da bi odstopilo (separiralo) del svojih gozdov podložnikom, da bi se ti na preostalih gozdovih odpovedali služnostnim pravicam. To se v obravnavanem času še ni posrečilo. Je pa problematika sorazmerno dobro obdelana. Na gospostvu Frajštanj separiranje gozdov za odstop služnostnih pravic v obravnavanem času še ni bilo aktualno. Na obeh gospostvih o sodobnem gospodarjenju z gozdovi še ni bilo sledu. Prav tako je bil na obeh gospostvih dohodek od gozdov neznaen.

In der Abhandlung werden die Wälder zweier Herrschaften Žiče (Seitz) und Frajštanj (Freistein) und ihre Bewirtschaftung in dem erwähnten Zeitraum behandelt. Beide wurden durch die Innerösterreichische Staatsgüteradministration in Gradec (Graz) verwaltet. Die erste Herrschaft lag im Hügelland um den Markt Konjice (Gonobitz), die zweite aber hauptsächlich in der Ebene um die Ortschaften Zgornja in Spodnja Polskava (Ober- und Unter Pulsgau), nicht weit voneinander entfernt. Die Wälder beider Herrschaften waren im großen Umfang mit Servitutsrechten zugunsten eigener Untertanen und Untertanen fremder Herrschaften belastet. Bei der ersten machte sich die Verwaltung Gedanken um in den Wäldern Separation durchzuführen, die eine bessere Bewirtschaftung der Wälder gewährleisten sollte, obwohl die Separation erst nach einigen Jahrzehnten durchgeführt wurde. Bei der zweiten Herrschaft wurde die Separation noch nicht in Betracht gezogen.

Die Herrschaft Žiče

Die Staatsherrschaft, bzw. genauer die Herrschaft des Steirischen Religionsfonds in Graz, bzw. Religionsfondsherrschaft Žiče, war rechtlich aus zwei Herrschaften zusammengesetzt. Die erste lag in Žiče, die zweite unweit in Žička vas (Seitzdorf), wurden aber immer schon zusammen verwaltet. Die erwähnte Herrschaft entstand 1782 durch Aufhebung des Kartäuserklosters in Žiče. Provisorisch ging sie in Besitz des Steirischen Religionsfonds und in die Verwaltung der Innerösterreichischen Staatsgüteradministration in Gradec über. 1794 wurde sie in definitives Eigentum des erwähnten Fonds überschrieben, verblieb aber noch weiter unter derselben Verwaltung bis sie 1828 Fürst Weriand Windischgrätz erwarb.

Kartäuserkloster in Žiče gründete der steirische Markgraf Otokar V. (1129-1164). Sein Schwager, der kärntnerische Graf Bernhard von Trušnje (Trixen), der als Kreuzritter im Heiligen Land 1147 verstarb, vererbte ihm Maribor (Marburg) und umfangreiche Besitzungen in der Untersteiermark, darunter auch die Umgebung von Konjice (später umbenannt in Slovenske Konjice), so auch das Tal an der Südseite der Konjiška gora (Gonobitzberg). Otokar V. bereiste früher Frankreich, wo er sich mit dem neuen Kartäuserorden bekannt machte und wollte so ein Kloster auch auf seinen Besitzungen gründen. Kartäusermönche waren aber zu diesem Schritt nicht gewogen. Schließlich gelang es ihm mit Hilfe des Papstes Alexander III., daß die Mönche 1160 nach Konjice kamen, wo sie im geräumigen Pfarrhof wohnten, bis sie sich im entlegenen und ruhigen Tal in Žiče den ersten provisorischen Kloster aufbauten. Das war der erste und später sehr bedeutende Kloster dieses Ordens im Bereich des Heiligen Römischen Reichs (später der deutschen Nation genannt).

Weil das Tal unter Konjiška gora damals zwar Otokars Eigentum, jedoch als Lehen dem Ritter Liupold von Konjice vergeben wurde, gab ihm Otokar für diese Abtretung andere Besitzungen. Kartäuserorden baut nämlich seine Klöster seit seiner Gründung

immer in entlegenen und einsamen Gegenden. Die Gründungsurkunde für das Kloster stellte der Sohn von Otokar V., Otokar VI., 1165 aus², der in Žiče auch begraben wurde. In den nächsten Jahrhunderten erwarb das Kloster durch Schenkungen zahlreicher Adliger, z. B. denen von Vojnik (Hocheneegg), denen von Konjice (die sie damit das erbliche Recht zur Bestattung der Toten ihres Geschlechts im Kloster zuerkannt bekamen), der Bischöfe von Krka (Gurk), der Liechtensteiner und später der Grafen (bzw. Fürsten) von Celje (Cilli)³ umfangreiche Besitzungen, darunter natürlich viele Waldungen. Die Herrschaft Oplotnica (Oplotnitz) umfaßte 4.509 Joch Waldungen⁴. Diese war aber nicht im Verband der Herrschaft Žiče und wurde extra verwaltet. Kloster Žiče hat sich nach den Türkeneinfällen soweit erholt, daß es vom Staat die ehemaligen dem Grafen Erasmus Tattenbach gehörigen Herrschaften Konjice, Trebnik, Zreški grad, Gojka und das Amt Zreče bei Konjice, die ihm wegen der Teilnahme an der Verschwörung der Grafen Zrinski und Frankopan beschlagnahmt wurden, aufkaufte.

Weil die Herrschaft Žiče, sowie auch andere Herrschaften und Güter nach der Übernahme in die Staatsverwaltung nicht die erwarteten Erträge abwarfen, diskutierten die entsprechenden Landesorgane (das Innerösterreichische Gubernium, der erwähnte Steirische Religionsfond, die Staatsgüteradministration) und Hoforgane (Hofkammer und andere Hofämter) über mögliche Verbesserungsmaßnahmen und auch den möglichen Verkauf dieser an Private und zwar per Lizitation. Dazu mußten aber die Herrschaften und Güter genau beschrieben werden, ihre Gerechtsame und Verbindlichkeiten aufgeführt, so weit möglich ihre Gründe vermessen und das Kaufangebot erstellt werden. Auf Befehl des Kaisers und des Erzherzogs Karl verordnete die Staatsgüteradministration in Graz den Verwaltern der Herrschaften, daß sie mit ihrem Personal die Beschreibung der verwalteten Herrschaften und Güter (sogenannte Güterbeschreibungen) erstellen mußten, von denen die ältesten als erstklassige Quellen zur lokalen Wirtschaftsgeschichte der Zeit vor etwa zwei hundert Jahren dienen können.

Die Gutsbeschreibung der Herrschaft Žiče⁵ enthält Bilanzen mit verschiedenen Daten für die Jahre 1793 bis 1802, die aber hier nur insoweit erwähnt werden, als sie sich auf Waldbau beziehen. So kann man sehr gute Einsicht in die Wirtschaft einer verhältnismäßig großer Herrschaft, wie die von Žiče damals war, in die Struktur ihrer Wirtschaftszweige und ihren Anteil in den Jahreseinnahmen erhalten. In der vorliegenden Abhandlung soll nur der Stand der Wälder und ihrer Bewirtschaftung anhand des Kapitels über den Waldbau⁶ der erwähnten Gutsbeschreibung von Žiče und anhand der Bilanzbeilagen dargestellt werden. Die Beschreibung der Waldungen und ihrer Bewirtschaftung ist im Original in Form der Antworten auf schematisierte

² Zelko, Ivan: Žička kartuzija. Ljubljana, 1984, 7-9.

³ Pirchegger, Hans: Die Untersteiermark in der Geschichte ihrer Herrschaften und Gülden, Städte und Märkte. Buchreihe der Südostdeutschen Historischen Kommission, Band 10, München, 1962, 147-148.

⁴ Maček, Jože: O gospodarjenju z gozdovi na državni gosposčini v Konjicah na prehodu iz 18. v 19. stoletje. Gozdarski vestnik **35** (1977) 141-149.

⁵ Historische Gutsbeschreibung der Religions Fonds Herrschaft Seitz und Seitzdorf etc. 1802. Steirisches Landesarchiv Graz, Karthause Seitz, Schuber 1, Heft 2.

⁶ Ibid. Von der Beschaffenheit der Waldungen, 107-152.

Fragen dargestellt. Verhältnismäßig detaillierte Angaben ermöglichen gute Rekonstruktion des damaligen Standes der Herrschaftswaldungen. Dieser wurde erstmals vorläufig beschrieben und geschätzt bei der Theresianischen Steuerrekтификаction nach 1748, zweitens aber wegen der vorgesehenen Josephinischen Urbarial- und Steuerregulierung in den Jahren 1783 bis 1786. Von dieser Katasteraufnahme lagen aber keine Mappen vor. Wegen der Besteuerung wurden damals auch die Jahreserträge geschätzt. Die Wälder waren bis 1802 noch nicht geodätisch vermaßen, ebenso waren sie noch nicht in Reviere und Abteilungen eingeteilt. Die Herrschaft hatte noch keinen fachlich geschulten Förster, sondern nur einen Forstknecht oder Forstbedienten.

Die Herrschaft besaß mehrere Waldkomplexe in gesamttem Ausmaß von 2.365 Joch im möglichen Jahresetat von 1 Klafter Eichen-, 40 Klafter Nadelholz und 343 Klafter Buchenholz. In den Buchenwäldern gab es kein Bauholz, wohl aber einiges zur Herstellung von landwirtschaftlichen Geräten. Die Waldkomplexe waren:

1) Im Wald *D o b r i č k o v c a* (696 Joch) war Buche die Hauptbaumart, nur ein Neuntel der Fläche war mit der Tanne bewachsen. Wegen übertriebener Abstockung in früheren Jahren war dieser Wald erschöpft; jährlich wäre nur eine Abstockung von 30 Klafter Buchenholz möglich. Wegen Entlegenheit war er für den Marktverkauf des Holzes nicht geeignet. Die Untertanen in der Nähe gelegener Dörfer hatten da Servitutsrechte zum unentgeltlichen Bezug von Brennholz und der Viehweide, die Untertanen zu Fuße des Bergs aber zum Ankauf von Brennholz. Der Herrschaft war bewußt, daß die Untertanen ohne dieser Rechte auf ihren kleinen Besitzungen nicht überleben könnten. Sie hatte aber auch kein Geld um diese Rechte aufzukaufen. Darauf könnten die Untertanen aber auch nicht eingehen, weil es in der Nähe keine Wälder gab, die sie als Ersatz kaufen könnten. Auch die im voraus bestimmte Menge des bewilligten Holzes (besonders des Brennholzes) kam nicht in Betracht, weil dazu forstlich eingerichtete Wälder nötig sind, die sich zum Kahlschlag eignen, das galt aber für die Wälder dieser Herrschaft nicht. Schlußendlich käme nur Separierung, Zuteilung bestimmter Flächen herrschaftlicher Wälder den Untertanen in Betracht, damit sich diese der Servitutsrechte entsagten. Diese Betrachtungen galten inhaltlich für alle Wälder dieser Herrschaft. Die Verwaltung der Herrschaft hat deshalb die Zuteilung von 496 Joch schlechterer Teile dieses Waldes an die Untertanen vorgesehen, um die Abtretung der Servitutsrechte zu erreichen. Dazu würde sich die Herrschaft noch eine jährliche Dominikalabgabe von 9 kr je Joch ausbedingen; dafür könnte sie jährlich 74 Gulden 24 kr einstreichen. Den besseren Teil dieses Waldes, im Ausmaß von 200 Joch, wollte sich die Herrschaft zur eigenen Bewirtschaftung vorbehalten. Jedoch verblieb es bei diesem, als auch bei allen anderen Wäldern dieser Herrschaft, alles beim alten bis 1828, als Fürst Weriand Windischgrätz die Herrschaft kaufte, und auch noch danach bis 1864, als die fürstlichen Förster begannen den Untertanen ihre alten Rechte zu schmälern. Es wurde ein Rechtsstreit angestrengt, der 1866 zugunsten der Untertanen entschieden wurde.⁷ Die Servitutsrechte der Untertanen zur Viehweide, Brennholz und anderem nötigen, vor allem Bauholz in den Wäldern, bereiteten der Herrschaft bei ihrer Bewirtschaftung viel Schwierigkeiten, deshalb wollte sie sich dieser mit der schon erwähnten Separierung eines Teil der Wälder an die Untertanen entledigen. Bessere Waldteile wollte sich die Herrschaft

⁷ Zelko, o. c. 65.

vorbehalten. Es verblieb aber, wie schon erwähnt, nur bei der Absicht. Die Frage der Entledigung der Servitutsrechte wird weiter unten noch behandelt.

2) Ausschließlich Buchenwald *T o v s t i v e r h* maß ebenso 696 Joch. Ein Sechstel waren Kahlflächen und steiniger Grund, mit Bäumen im Alter von 10 bis 20 und von 20 bis 30 Jahren bewachsene Flächen waren je ein Sechstel, von 30 bis 40 Jahren zwei Sechstel und ein Sechstel waren zur Abstockung geeignete Bestände. Die Servitutsrechte waren gleich wie beim vorigen Wald. Möglicher jährlicher Schlagetat war um 120 Klafter. Zur Separierung wegen der Servitutsrechte waren 296 Joch vorgesehen, 400 Joch sollte in eigener Bewirtschaftung verbleiben. Aus diesem Wald wäre Brennholzverkauf in Konjice möglich, teilweise könnte aber das Holz im Bach *Žičnica* bis *Žička vas* getriftet werden, wo ein Holzlager eingerichtet werden müßte. Der Bach müßte dazu allerdings reguliert werden.

3) Der Wald *M a č k o v e c* war der beste herrschaftliche Wald. Er war bis zu zwei Dritteln mit Tannen, zu einem Drittel mit Buchen bewachsen. Er maß 530 Joch. Zur Abstockung geeigneter Bäume gab es ein Sechstel, unter 20 Jahren ein Sechstel, von 20 bis 40 und von 40 bis 60 Jahren je zwei Sechstel. Möglicher jährlicher Holzschlag war um 40 Klafter. Dieser Wald war mit Servitutsrechten zur Viehweide und Buchenholz, jedoch nicht zu Nadelholz belastet. Den Untertanen sollten zur Entledigung der Servitutsrechte 80 Joch des Waldes mit angrenzenden Weidegründen abgetreten werden, womit auch günstige Grenzziehung erreicht würde. Die Untertanen sollten dafür je Joch 15 kr bezahlen, das würde eine jährliche Dominikalgabe von 20 Gulden bedeuten. Auf den verbliebenen (eigenen) 450 Joch würde man nach und nach Buche abschlagen und sie in Monokultur der Nadelbäume umwandeln. Fichtenholz wurde gut als Bauholz verkauft, daraus wurden auch Weinbergstecken hergestellt. Dieser Wald entsprach zur schnellen Einrichtung der Abteilungen.

4) Kiefernwald *N a d V i r t a m n a K r a j n i* (7 Joch) war früher der Herrschaft zur Reparierung der Uferteiche, wenn es nötig war, vorbehalten. Jährlicher Holzschlag um zwei Klafter. Dieser Wald sollte ganz gegen 20 kr je Joch jährlich an die Untertanen abgetreten werden. Die jährliche Dominikalabgabe wäre 2 Gulden 20 kr.

5) Buchenwald *P l a t* (72 Joch) war bis zur Hälfte kahle Fläche, in der anderen Hälfte mit Bäumen im Alter von 20 bis 40 Jahren bewachsen. Er lag zwischen den Untertanenbesitzungen. Er diente ihnen zur unentgeltlichen Nutzung mit Holz und Viehweide. Nur wenige Bäume waren zur Abstockung geeignet. Möglicher jährlicher Schlag war um 36 Klafter. Dieser Wald soll zur Gänze an die Untertanen verteilt werden. Jährliche Abgabe je Joch wäre 15 kr, die jährliche Dominikalabgabe wäre also 18 Gulden 15 kr. Die Angaben für den Wald *Plat* gelten auch für den 6) Wald *G o l i r e b e r* (5 Joch). Dieser war eigentlich Gebüsch auf unfruchtbaren steinigem Boden, nur hie und da stand eine Buche. Er war mit dem Servitutsrecht der Viehweide behaftet. Er sollte ebenso verteilt werden. Hier könnte man jährliche Abgabe von nur 9 kr je Joch verlangen, die jährliche Dominikalabgabe würde also nur 45 kr (versehentlich ist 1 Gulden 30 kr eingeschrieben) betragen. Alles hier angegebene galt auch für 7) den Wald *L e p o g l a v a* (34 Joch), nur daß er aus noch mehr Kahlflächen bestand und die Bäume älter waren. Die Abstockung war nicht

vorgesehen bzw. möglich. Vom Joch könnte man von den Untertanen nur 12 kr verlangen, so wäre die jährliche Dominikalabgabe 7 Gulden. Ungefähr dasselbe galt auch für den 8) Buchenwald Š u m e n c a (30 Joch). Er war mehr Gebüsch als Wald und mit Servitutsrechten zu Brenn- und anderem Holz behaftet. Möglicher jährlicher Holzschlag war 7 Klafter. Für ein Joch konnte von den Untertanen 15 kr verlangt werden, jährliche Dominikalabgabe wäre als 7 Gulden 45 kr. Etwas besser war der zwischen Untertanenbesitzungen liegende 9) Wald S v i b o v e c (13 Joch), sonst war aber alles gleich wie bei den bisher erwähnten. Jährlich war Abstockung von 3 Klaftern möglich. Die jährliche Untertanenabgabe wäre je Joch 15 kr, abgerundete Dominikalabgabe betrüge also 3 Gulden.

Von den beiden vorigen war besser 10) der Wald Z l o d e r š n i k (256 Joch), bewachsen mit Bäumen ziemlich gleichen Alters. Mögliche jährliche Abstockung war um 128 Klafter. Er war mit Servitutsrechten für Holz und Brennholz behaftet. Für die Entledigung erwähnter Rechte würde man den Untertanen 56 Joch je 15 kr abtreten, das ergäbe jährliche Dominikalabgabe von 14 Gulden. Mit dem verbliebenen Waldteil würde die Herrschaft selbst wirtschaften und ihn in Abteilungen einteilen. Darin würde man vor allem Brennholz gewinnen, die man an den Holzlager in Loče befördern würde, worüber ein dortiger Untertan gegen mäßige Bezahlung die Aufsicht übernehmen würde. Dies würden heimische Untertanen mit billiger Herstellung von Brennholz und mäßigen Transportkosten ermöglichen, gleichzeitig hätten sie aber auch Beschäftigung in der Zeit als es keine dringende landwirtschaftliche Arbeiten gibt. In dieser Gegend, die von dem Wald nur eine gute Stunde entfernt ist, sollte nämlich in den nächsten Jahren Holzangel entstehen.

11) Kleiner Wald N a V r e s j u (2 Joch) war mehr Gebüsch und Weide als Wald. Es war nichts abzustocken. Er war zur Zuteilung an die Untertanen vorgesehen, wofür die Herrschaft eine Dominikalabgabe von 3 Gulden einstreichen würde. 11) Der Eichenwald N a D o b r o v i p r i Z a f o r š t i (2 Joch) lag in der großen Gemein Dobrovka. Weil er mit den Viehweideservituten behaftet war, sollte er den Untertanen gleichzeitig mit der angrenzenden Weide zugeteilt werden. 1802 war der Wald ungefähr 40 Jahre alt. In der hiesigen Gutsbeschreibung steht, daß er mit Eichensetzlingen (...von verpflanzten Eichen...) bepflanzt wurde, was sicher ein seltener Fall der Bepflanzung von Hartholzarten, und erst recht von Eichen, in dieser Zeit darstellte. Es wäre möglich pro Jahr eine Klafter abzuschlagen. Die Herrschaft würde sich bei der Zuteilung an die Untertanen für die Abtretung der Servitutsrechte das Recht der ersten Abstockung vorbehalten. Dominikalabgabe der Untertanen betrüge jährlich 6 Gulden.

12) Buchenwald - Weide K e r č i č n a k z a H o m c a m (16 Joch) war mittelmäßig mit Bäumen mittleren Alters bewachsen. Jährlich wäre 15 Klafter zu schlagen möglich. Er war mit den Servitutsrechten zur Weide und Holz behaftet. Dieser war in Gänze für die Zuteilung den Untertanen vorgesehen. Von Joch könnte die Herrschaft 12 kr verlangen, die Gesamtdominikalabgabe betrüge also 3 Gulden 24 kr.

Alle Gemeinweiden im Gesamtausmaß von 173 Joch sollten ebenfalls verteilt werden. Die Gemeinweide N a D o b r o v i, im Ausmaß von 80 Joch sollte in Äcker umgewandelt werden. Die Einnahmen dafür sind aber in einem anderen Abschnitt dieser Gutsbeschreibung dargestellt. Die Gemeinweiden im Ausmaß von 93 Joch

sollten unter die Untertanen verteilt werden. Für ein Joch konnte 1 Gulden verlangt werden, also wäre die Einnahme der Herrschaft 93 Gulden. Insgesamt würden die Dominikaleinkommen von den Wäldern ungefähr 313 Gulden 25 kr betragen. Bisherige Abstockung von 966 österreichischen Klafter für Brennholz (auf dem Stock), betrug je Klafter 15 kr und die Einnahme davon 242 Gulden 30 kr, die natürlich die Untertanen zur Gänze bezahlen mußten. Die Viehweide würde in den verbliebenen herrschaftlichen Waldungen zur Gänze abgeschafft. So würden sich die verkleinerten Wälder für die Herrschaft mehr rentieren als die bisherigen großen.

Für die Herrschaft von Žiče war, solange sie in Besitz des Steirischen Religionsfonds war, charakteristisch, daß sie teilweiser Abtretung (Separierung) der Wälder zur Entledigung der Servitutsrechte, wie aus den archivalischen Quellen ersichtlich, gewogen war. Die Gründe für die vorgesehene Abtretung der Wälder waren folgende:

- 1) Die Herrschaft sah vor, daß für die Zuteilung an die Untertanen nur schlechtere Wälder in Betracht kämen, für welche sie sogar selbst erklärte, daß sie nur zur Schafweide geeignet sind, behalten wollten sie aber nur gute und aus der Transportsicht günstigere Wälder;
- 2) Nur auf diese Weise könnte sie einen Teil der Wälder für ihre vollkommen freie Bewirtschaftung gewinnen;
- 3) Bisher uneinträgliches Eigentum würde für die Herrschaft einträglich werden (...Die Herrschaft erhält aus ihrem bisher unfruchtbaren Eigentum einen jährlichen baaren Ertrag an Steuern...)⁸
- 4) Die Herrschaft hat außer der vorgesehenen Verteilung schlechterer Wälder an die Untertanen für die Abtretung der Servitutsrechte keine andere Mittel in den Händen, um ihr Eigentum zu befreien und in der Zukunft mit ihm frei zu wirtschaften;
- 5) Solange die Untertanen noch Miethuben besaßen und die Herrschaft darüber nur das Obereigentum (*Dominium directum*) ausübte, konnte jeder Untertan aus den herrschaftlichen Wäldern nehmen was er bedurfte. Seit die Miethuben in kaufrechtliche Huben umgewandelt wurden, werden ihnen in strittigen Fällen, nach hoher Entscheidung aus dem Jahre 1788, alle Pertinenzen die ehevor zu den Huben gehörten, zuerkannt. In strittigen Fällen würde die Herrschaft ihre vermeintlichen Rechte verlieren.
- 6) Für die Herrschaft entfällt vollkommen die Gefahr, daß sie bei künftigen Steuerregulationen von uneinträglichem Eigentum Steuern zahlen mußte.
- 7) Für das Allgemeinwohl und das Wohl der Untertanen ist die Separierung der Wälder besser, weil diese echtes Eigentum mehr schätzen als Servitutsrechte an herrschaftlichen und Gemeingründen.
- 8) Durch Separierung der Wälder geschieht viel leichter die Umwandlung geringwertiger Wälder in Weiden, Gemeinweiden in Wiesen, Gemeingründen in Äcker. Auch ist die allgemeine Aufteilung der Gemeingründe erleichtert.
- 9) Entfallen würde reichlicher Verbrauch von Holz für Umzäunungen, die bei der Dreifelderwirtschaft notwendig sind.

Von den Wäldern im Ausmaß von 2365 Joch sollte die Herrschaft 1115 Joch an die Untertanen verteilen, selbst wollte sie 1250 Joch besserer Wälder behalten. Diese Aufteilung wäre für die Untertanen natürlich nicht umsonst, da sie für Joch von 9 bis

⁸ Historische Gutsbeschreibung...Seitz, 133.

15 kr jährlich Dominikalgabe zahlen mußten. Das wäre aber für die Herrschaft einträglicher, da sich bisher die Einnahmen für Holz im Durchschnitt von 9 Jahren (1793-1801) auf 87 Gulden beliefen.⁹ Die herrschaftlichen Wälder verjüngten sich selbst; es wurden keine Samen gesät und keine Setzlinge gesetzt. Damit im Gegensatz ist die Angabe beim Wald Na Dobrovi pri Zaforšti, der angepflanzt wurde. Die Angabe über diese Pflanzung müßte mit anderen Archivquellen überprüft werden. Jedenfalls handelt es sich um eine seltene Forstmaßnahme in dieser Zeit. In den Wäldern der untersteirischen Herrschaften Jurklošter, Konjice, Marenberg, Studenice und Štanof, die aus forstgeschichtlicher Sicht eben für diese Zeit schon behandelt wurden, ist Bepflanzung mit Laubblattsetzlingen nicht erwähnt. Außer dieser Ausnahme waren alle Wälder Plenterwälder. In hiesigen Wäldern wurden auch keine Samenbäume belassen. Waldbrände gab es nicht, außer einmal vor längerer Zeit im Wald Dobričkovca, in steiler Lage und auf steinigem Grund. Nachbarschaftliche Gemeinden waren verpflichtet beim Löschen der Brände zu helfen. In den Wäldern war Rauchen von Tabak und Anstellen von Hirtenfeuern streng untersagt. Solche Übertretungen wurden nie ermittelt.

Nutzung der Wälder für Brennholz, für anderes Holz und für Viehweide konnte die hiesige Herrschaft den Untertanen nicht wehren, weil sie auf uraltem Recht gründete. Jedoch verblieben in den Wäldern nach dem Fällen dünnere Äste und Reisigholz, die ein wenig das Vieh abwandten und kleines Unterholz schützten. Zum Brennholz für eigenen Bedarf waren 160 Untertansfamilien und der Pfarrer in Češnjice berechtigt. Zeitlich und zahlenmäßig unbegrenzte Viehweide durften der erwähnte Pfarrer und 186 Untertansfamilien nutzen. Die Untertanen konnten für ihre Nutzungen in den Wäldern keine besonderen Lagen beanspruchen. Die Servitutsrechte waren nach der Ansicht der Herrschaft rechtmäßig und stammten noch aus Zeiten ab, als die Wälder noch keinen Wert darstellten, und diese, die sie rodeten sogar belohnt wurden (... wo das Gehölz nicht nur keine Wert hatte, sondern noch Belohnungen jener warteten, die Wälder aussrotteten...)¹⁰ Die Untertanen, die zu den Nutzungen in den Wäldern berechtigt waren, leisteten für ihre Berechtigung Hafer *in natura*, der koplevnik hieß. Dieser ist aber in der Gutsbeschreibung unter Zinsgetreide beschrieben. Die Untertanen, die zur Nutzung von Brennholz aus dem Wald Dobričkovca berechtigt waren, leisteten ähnliche Abgabe sogar fremder Herrschaft, und zwar der Gutsherrschaft Zalog bei Žalec. Das zeigt, daß diese Herrschaft vor langer Zeit den erwähnten Wald dem Kloster Žiče schenkte, sie hat sich aber die Abgabe der Untertanen vorbehalten. Es ist nicht ausgeschlossen, daß koplevnik auch Abgabe für Weiderechte war.

Bei der Betrachtung über Möglichkeiten der Begrenzung des Fällens von Buchenholz als Brennholz der Untertanen willigte die Herrschaft schließlich auf 6 bis 8 Klafter je Familie ein, dies sollte aber nur Zusatz zu dem Holz sein, das die Untertanen in ihren eigenen Wäldern fällen konnten. Großes Hindernis zur rationalen Bewirtschaftung der Wälder war seit undenklichen Zeiten bestehendes Recht, daß die Untertanen sich das nötige Holz in herrschaftlichen Wäldern selbst auswählen konnten. Die Herrschaft wollte unentgeltliche Anweisung des Holzes einführen. Diesem Vorhaben haben sich die Untertanen scharf entgegengesetzt, weil sie seit Urzeiten das Holz nach eigener

⁹ Ibid., 147

¹⁰ Ibid., 118

Auswahl, ohne Einmischung der Herrschaft, fällen durften. Hinsichtlich des Standes, den die damalige Herrschaftsverwaltung (seit 1782) fand, schien es, daß Mißbrauch nach damaligen Vorschriften in Wirklichkeit ein uraltes Recht war.

Wegen großer Unzufriedenheit, die unter den Untertanen gegen Ende des 18. Jahrhunderts wegen nicht normaler Belastungen in den Kriegen, durch obligate Requisitionen der Lebensmittel, Vorspann bei militärischen Transporten, obligate Einquartierung des Militärs, größere steuerliche Belastungen usw. verbreitet war, meinte die Herrschaft, daß es nicht klug wäre, die Neuheit der Holzanweisung mit Zwangsmitteln zu erzwingen, sondern es klüger sei, zunächst nachgiebig zu sein und diese allmählich durchzuführen bzw. sie auf spätere Zeiten zu vertagen. Die Herrschaft verschaffte sich aber ziemlich genaue Übersicht, wieviel Holz aus den herrschaftlichen Wäldern die Untertanen für sich abstockten. Wahrscheinlich ergab diese Übersicht, daß ein einzelner Berechtigter um 6 Klafter Holz fällt. Das ergäbe etwa 966 Klafter Holz pro Jahr.

Weideservitute waren weder nach Art des Viehs noch ihrer Zahl und Zeit begrenzt.

Wenn man den zahlenmäßigen Stand des Viehs bei den Untertanen unter der Voraussetzung, daß alles Vieh geweidet würde (was bestimmt nicht zutraf), wären es etwa 1168 Stück. Neben der Waldweide hatten die Untertanen das Recht der Nutzung von 173 Joch der Gemeinweide, auf der auch diese Untertanen weiden durften, die kein Recht zur Waldweide hatten, oder waren von den Wäldern zu weit entfernt. Weiderechte an der Gemeinweide wurden nach Ortschaften hinsichtlich der Nähe zu dieser geltend gemacht. Interessant ist die Angabe, daß Weideservitutsrechte Belastung der herrschaftlichen Gründe aus neuerer Zeit seien und daß sie früher von dieser Belastung frei waren (...ihr vormals vollkommenes Eigentum zu besitzen...). Das ist natürlich die reinste Fiktion, die aber zur behandelten Zeit bei den Gegnern der Regulierung der Servitutsrechte und bei den Grundherren sehr verbreitet war, und hat sie auch der Verwalter dieser Herrschaft in der Gutsbeschreibung niedergeschrieben. Natürlich war die Wirklichkeit ganz anders. Die Nutzung der Gründe war in der Vergangenheit noch wesentlich mehr verflochten als zu Ende des 18. Jahrhunderts, da ja die späteren primären Wirtschaftszweige Land- und Forstwirtschaft noch nicht getrennt waren. Es bestand nur eine alles umfassende Wirtschaft. Es stimmt aber, daß die Wirtschaftsdoktrin des ausgehenden 18. Jahrhunderts die einseitige Nutzung von Grundkategorien empfahl: Wälder für Holz, Weiden zur Viehweide, Äcker zum Anbau der Feldfrüchte, und vor allem, nur der Eigentümer sollte allein das Recht haben seine Gründe zu nutzen. Dabei dienten ihnen als warnendes Beispiel besonders die Gemeinweiden. Diese hatten zwar einen nominellen Eigentümer, die Herrschaft, aber keine richtige Verwaltung. Sie waren meistens im schlechten Zustand, weil darauf wegen der Geltendmachung der Weiderechte verschiedener Berechtigten, mit sehr verschiedenen Interessen, keine geordnete Bewirtschaftung, die Nachhaltigkeit sichern könnte, möglich war. Natürlich behinderten aber die Servitutsrechte die gute Bewirtschaftung der herrschaftlichen Gründe. Der Hauptgrund für das Bestehen der Servitutsrechte sollte die zerstreute Lage der Ansiedler, ebenso wie die zerstreute Lage der herrschaftlichen Gründe sein. Der zweite Grund sollte aber die niedrige Bewertung aller Gründe außer den Äckern sein (...in der ursprünglichen Geringhaltung alles dessen was nich Ackerland war...)¹¹

¹¹ Ibid., 119.

Die Verwaltung der Herrschaft Žiče hatte große, zwar übertriebene Angst, daß die Untertanen ihre, schon von vorher eigene und neu zugewonnene Wälder wegen Holzmangels zu stark ausnützen und damit Devastationen verursachen könnten. Daß diese Möglichkeit verhindert würde, hat sie einen Vorschlag ausgearbeitet, wonach jeder Untertan wenigstens auf einem Joch seines Waldes obligat nach den Anleitungen der Herrschaft wirtschaften müßte. Er sollte dieses Joch mit schnellwüchsigen Pappeln bepflanzen. Damals kannte man schon Klone, die auf sehr unfruchtbaren Böden gedeihten und diese sollte man auf der Herrschaft von Žiče anpflanzen. Diese Baumart konnte man vegetativ mit Setzlingen vermehren (ihre Gewinnung ist beschrieben), sie war einträglich, vom Joch konnte man etwa 300 Stämme ernten, 7 oder 8 aber würden genügen für die Beheizung eines Untertanenhaushalts. So würde man aus dem Brennholz wertvollere Baumarten aussondern. Von der Pappel konnten auch Blätter zur nötigen Streu gewonnen werden. Der Vorschlag sollte auch deshalb anregend sein weil man den Umtrieb auf 30 Jahre verringern könnte, das ist aber eine Zeitspanne die sie mancher Grundbesitzer noch selbst erlebt und so die Frucht seiner Arbeit genießen könnte.

Weil die Untertanen auch eigene Wälder besaßen, gab es in den herrschaftlichen Wäldern keine Diebstähle. Die Untertanen haben auch nicht die Bäume beschädigt, sie waren ja interessiert, daß die Wälder wo sie Servitutsrechte besaßen, in gutem Zustand waren. Die Bäume fällte man so, daß man ein Schuh hohen Stock beließ. Die Stöcke wurden nicht ausgegraben, da Holz in diesen Wäldern keinen so hohen Preis hatte, daß sich das auszahlen würde. Die Stöcke wurden auch nicht mit Erde bedeckt, damit sie schneller vermoderten. In der Gutsbeschreibung stand auch eine Frage ob Nichtsnutze die Wipfeln der Bäume abhackten. Das ist in dieser Herrschaft nie vorgekommen. Hier gab es auch keine geschlossene Birkenwälder. Birkenäste wurden zur Verfertigung von Birkenbesen abgehackt, die Untertanen, die auch Wagner waren, besorgten sich Birkenholz zur Verfertigung von Radteilen aus eigenem Wald, oder kauften es. Die Holzberechtigten machten sich im Falle des Futtermangels Astfutter, "vejnik", sie hackten im Herbst dünnere Äste mit viel Laub ab, trockneten sie und verfütterten sie im Frühling ihrem Vieh. Es ist aber interessant, daß die Gewinnung der grünen Streu mit Abhackung der Sträucher auf den Wiesen oder am Waldesrand vom späten Frühling bis späten Herbst, die in der Untersteiermark mancherorts bis unlängst verbreitet war, hier nicht erwähnt ist. Die Untertanen hatten seit Urzeiten das Recht im Herbst in den Wäldern abgefallenes Laub als Streu zusammenzurechnen und nach Hause zu fahren. In den herrschaftlichen Wäldern gab es keine geheime Wege, es hielten sich in ihnen keine Vagabunden auf. Keuschen, die sich in den Gereuten dieser Wälder befanden, wurden monatlich von den Polizisten kontrolliert, einmal jährlich inspizierte sie auch der Gemeinderichter.

Die Herrschaft verkaufte Holz nur auf dem Stock. Für eine Klafter war der Preis im Jahre 1802 15 kr. Ein Stamm mit 6 Klaftern kostete also 1 Gulden und 30 kr. Dünnerer Tannestamm kostete 20, mittlerer 40 kr und dicker 1 Gulden. Größerer Tannestamm für Weinbergstecken kostete 1 Gulden 30 kr. Dünnerer Eichenstamm kostete 40 kr, mittlerer 1 Gulden, dickerer aber 2 Gulden. Eichenstamm mit ausgezeichnetem Wuchs für Faßdauben kostete 6 Gulden. Dauben, die sie die Herrschaft ausnahmsweise auch selbst herstellte, kosteten je Stück 4 kr. Jedoch gab es Eichenbäume mit entsprechenden Stämmen sehr wenige. Die Käufer konnten Holz aus den herrschaftlichen Wäldern ausschließlich nur mit schriftlichen Anweisungen

erhalten. Dies konnte die Herrschaft gegenüber heimischen Untertanen, die zum Holz berechtigt waren, nicht durchsetzen. Weil die Herrschaft keinen eigenen Förster hatte, stellte der Verwalter den Käufern schriftliche Anweisung, worin die Zahl der Stämme, ihre Qualität und Lage im Wald, wo sie gefällt werden sollten, beschrieben war. Damit sprach der Käufer beim Forstknecht vor, der diesem die Bäume anwies und sie kennzeichnete, er definierte auch die Qualitätsklasse und stellte die Rechnung aus. Danach empfing er die entsprechende Geldsumme, die er bei der Herrschaftskasse abgab. Wesentlich war, daß der Forstknecht die Bäume nur gegen schriftliche Anweisung des Verwalters anweisen konnte. Damit war die Möglichkeit der Veruntreuung beim Holzverkauf zu Schaden der Herrschaft verhindert. Der Käufer mußte das Holz nur auf bestimmten Waldplätzen zusammenführen, damit Kontrolle möglich war. Die Herrschaft verkaufte in 8 Jahren 51 dünnere, 12 mittlere und 2 dickere Eichenstämme, 325 dünnere, 284 mittlere und 262 dicke Buchenstämme, 330 dünnere, 261 mittlere und 75 dickere Tannenstämme (darunter waren auch einige Kiefernstämme) und 20 Tannenstämme für Latten. Aus dem Tannenholz wurden Weinbergstecken, geringe Zahl von Brettern und Stangen hergestellt. Für die Herrschaftsgebäude wurde einiges Bauholz verwendet. Außerdem bekamen Bedienstete aus dem Wald ihr Holzdeputat. Das Holz wurde meistens den Bürgern von Konjice, in geringem Umfang auch fremden Untertanen in Loče verkauft.

In den Buchen- weniger in den Eichenwäldern war in den Jahren als diese Baumarten fruchteten, die Schweinemast üblich. Jedoch war die Fruchtbarkeit der Eichen und Buchen sehr variabel. Im Durchschnitt von 9 Jahren war die Einnahme von Schweinemast 36 Gulden. Im Jahre 1794 belief sich die Einnahme auf 113, 1797 aber auf 166 Gulden, für das Holz aber in eben diesen Jahren 148 bzw. 36 Gulden, jedoch waren die Einnahmen für Holz mehr beständig.

Die Herrschaft besaß nur die niedrige Jagd, die in Pacht ausgegeben wurde. Es bestanden drei Jagdreviere, das erste war im Wald Zloderšnik in der Pfarre Loče bei Poljčane, das zweite in den Wäldern, die in den Pfarren Loče und Črešnjice lagen, das dritte in den Wäldern die in der Pfarre Dramlje bei Šentjur lagen. Die durchschnittliche Jagdpacht betrug jährlich 49 Gulden.

Weil die Herrschaft bis zum Jahr 1802 ihre Wälder nicht modern bewirtschaftete, hatte sie mit ihnen auch keine spezifischen Unkosten. Es war auch kein Forstetat vorgesehen. Die Übersicht über das verkaufte Holz wurde wegen geringer Mengen sehr einfach geführt, die von der Staatsgüteradministration bewilligt wurde. Der Übergang auf mehr zeitgemäße Wälderbewirtschaftung gelang noch nicht. Die herrschaftlichen Wälder waren geldlich noch unrentabel. Wenn durchschnittliche jährliche Einnahmen für Holz, 87 Gulden, und für Schweinemast, 36 Gulden, zusammen- und Gehalt für den Forstknecht, 100 Gulden mit Holzdeputat, abgezogen wird, verbleibt ein unbedeutender Betrag. Wenn man noch die Wälderverwaltung (verhältnismäßiger Anteil des Verwaltergehalts) berücksichtigte, so zeigte sich der Waldbau als Wirtschaftssparte vollkommen unrentabel.

Die Herrschaft Frajštajn

Zwischen Zgornja in Spodnja Polskava (Ober- und Unter Pulsgau) stand das Schloß Frajštajn (Freistein). In den mittelalterlichen Quellen wird es noch nicht erwähnt. Seine Besitzgeschichte in dem Ortslexikon des Drauer Banats (1937)¹² und der Geschichte Untersteirischer Herrschaften, Gülten, Städte und Märkte von Pirchegger¹³ unterscheidet sich ziemlich stark. Wir entschieden uns für die Ausführungen des letzteren. Im Dorf Sele (Czell) besaß Albert Feistritzer, als Lehen der Grafen von Celje, zwei Huben und zwei Hofstätten. Dieser Besitz übergang 1445 auf Erasmus von Wilthaus und Walter Safner, im 16. Jahrhundert an die Regall zu Rače (Kranichsfeld) und Polskava, die wohl den Sitz (Schloß) erbauten und ihren Anteil an dem Erbe von Gromperg (Grünberg) hierher verlegten. 1603 verkaufte Frau Anna Regall, geborene Haller, die Herrschaft Frajštajn dem Georg von Stubenberg, dann folgte Erasmus von Dietrichstein. 1635 haben aufständische Untertanen das Schloß zerstört. Erasmus von Dietrichstein kaufte von Siegmund von Stubenberg, Hans Galler, Margarethe Wagen, geborene Rab, und von Gregor Matetitsch Gülten auf. Bei der Teilung des Familienbesitzes 1671 erhielt Graf Sigmund Hellfried von Dietrichstein Frajštajn, geschätzt auf 7.123 Gulden 31 ½ Pfund mit Wildbann, Raisgejagd und Landgericht, die Fischerei zwischen Zgornja und Spodnja Polskava, sowie die Vogtei über die Kirche in Spodnja Polskava. Bereits 1679 verkaufte er die Herrschaft dem Nonnenkloster Studenice (Studenitz) um 24.000 Gulden. 1754 besaß die Herrschaft 154 untertänige Häuser, 1782, als das Dominikanerinnenkloster aufgehoben wurde und in Staatsverwaltung übergang, aber 206 in zwei Ämtern mit allem Zugehör (doch gemeinsam mit den Gütern Zgornja Polskava, Gromperk und Vartenhajm (Wartenheim). 1828 kaufte dann die Herrschaft vom Steirischen Religionsfond Graf Clemens Brandis, 1904 dann Graf Batthyany, der auch Vartenhajm und die Gült Gromperk erworben hatte. Bei der Grundentlastung nach 1848 war die Ablöse 66.429 Gulden.

Diese Herrschaft besaß zu Ende des 18. Jahrhunderts 14 Wälder, die 1423 14/64 Joch und 19 Quadratklafter (Qk) maßen¹⁴. Sie waren mit Laub- und ein wenig mit Nadelbäumen bewachsen. Bis 1796 waren die Wälder noch nicht in Reviere und Abteilungen eingeteilt. Die Abstockung geschah nicht mit Kahl-, sondern mit dem Plenterschlag. Im Wald wurde hie und da zum Fällen geeigneter Baum ausgewählt. Die Bäume wurden für die Bedürfnisse der Herrschaft im Spätherbst und im frühen Winter abgestockt. Die Bäume die überflüßig schienen und sie ohne Schaden für den Wald geschlagen werden konnten, wurden von unabhängigen Schätzern geschätzt und wurden per Lizitation an Meistbietende verkauft. Mit dieser Abstockungsart entstand zwischen den Bäumen, geeigneten zur Abstockung und Jungwuchs ein Verhältnis, demnach kein Holzangel zu befürchten war. Nach den Berechnungen, die für die Steuer- und Urbarialregulation (1786-1789) gemacht wurden, könnte ruhig 246 Klafter Holz geschlagen werden.

¹² Krajevni leksikon Dravske banovine. Ljubljana, 1937, str. 423.

¹³ Pirchegger, 127.

¹⁴ Oekonomische Beschreibung der k. k. Staats, und Religions Fonds Herrschaft Freystein in Untersteier. Freystein den 1ten 7ber 1796. Hofkammerarchiv in Wien, Staatsgüterakten, Fasz. rote Nr 18. Die Antwort auf die Frage 11.

Die Wälderbeschreibung. 1) Der Wald *V e l e n i k* grenzte mit dem Wald der Herrschaft Rače und dem Gut Pragersko (Pragerhof). Er maß 114 $\frac{6}{4}$ Joch und 170. Darin wuchsen Laub- und Nadelbäume. In diesem hatten die Gemeinden Sele und Pokošė unentgeltliches Weiderecht, alle anderen Nutzungen, inklusive der Schweinemast gehörten der Herrschaft Frajštanj. Sie war berechtigt ihr Vieh überall dort zu weiden, wo die erwähnte Gemeinde das Dorfviehweiderecht hatte.

2) Der Erlenwald neben dem Teich von *V e l e n i k* war Gemeineigentum der Herrschaften Frajštanj, Zgornja Polskava, Rače, des Gutes Pragersko und der Dorfgemeinde Nova vas. Er maß 16 $\frac{6}{64}$ Joch und 5 Qk. Unentgeltliches Weiderecht genossen Dorfgemeinden Nova vas und Pokošė. Die Erlenbäume wurden, wenn sie zur Abstockung reif waren, auf gemeinsame Kosten geschlagen, danach aber in fünf Teilen den erwähnten Eigentümern gegeben. Diese Abstockung wurde 1788 zum letztenmal durchgeführt.

3) Der Erlenwald unter dem Teich von *V e l e n i k*, beim Auslauf des Baches, war ebenfalls Gemeineigentum der Herrschaften Frajštanj, Rače, Zgornja Polskava und dem Gut Pragersko. Er maß 5 $\frac{40}{64}$ Joch und 16 Qk. Die Dorfgemeinde Nova vas genoß das unentgeltliche Weiderecht, die Holzberechtigung teilten sich zu gleichen Teilen die erwähnten Dominien. Von den Wäldern uner 2) und 3) bestanden geodätische Mappen.

4) *S t a r i g o z d* wurde nur von Erlen bewachsen. Er lag zwischen dem Wald der den Herrschaften Rače und Zgornja Polskava gehörte. Er maß 195 $\frac{25}{64}$ Joch und 25 Qk. Darin hatten Weidrechte gegen Entgelt Dorfgemeinden Leskovec, Vrhloga und Črešnjevėc. Die erste Dorfgemeinde bezahlte seit jeher 5 Gulden 54 kr, die zweite 3 Gulden 54 kr, Črešnjevėc 3 Gulden 53 kr, 2 Pfenige. Alle andere Waldnutzungen gehörten der Herrschaft Frajštanj.

5) Im Wald *O s r e d e k* wuchsen nur Erlen und Eichen. Er maß 67 $\frac{24}{64}$ Joch und 17 Qk. Darin hatte die Dorfgemeinde Vrhloga unentgeltliches Weiderecht, alle anderen Rechte, auch der Schweinemast gehörten der Herrschaft Frajštanj. In diesem Wald wuchsen Erlen, die man am günstigsten für "Gespere" je 6 kr und für Latten je 3 kr verkaufen konnte.

6) Im Wald *D a l e* wuchsen ebenso Erlen und Eichen. Darin hatten die Dorfgemeinden Ternovec und Brezje unentgeltliche Weidrechte. Alle anderen Rechte gehörten der Herrschaft Frajštanj, besonders ergiebig waren in einigen Jahren die Einnahmen aus der Schweinemast.

7) Im Walde *G a j i č* wuchsen ebenso Erlen und Eichen. Er maß 9 $\frac{45}{64}$ Joch und 15 Qk. Auch in diesem Wald hatte die Dorfgemeinde Brezje unentgeltliches Weiderecht. Die Rechte für Holz, Schweinemast und Streu aber genoß die Herrschaft Frajštanj.

8) Der Erlenwald in *Č r e t* maß 68 $\frac{33}{64}$ Joch und 14 Qk. In diesem Walde genoß alle Rechte die Herrschaft Frajštanj. Aus jungen Erlen dieses Waldes wurden günstig Gespere und Latten hergestellt, zu Preisen, die oben angegeben sind. Die Dorfgemeinden Trnovec, Brezje und Sestrše wollten das Recht zur unentgeltlichen

Viehweide geltend machen, das aber die Herrschaft nicht zugestand und darüber ein Prozess angestrengt wurde.

9) Der Wald *P r e v r a t* maß 157 28/64 Joch und 15 Qk, 10) der Wald *H u d a m l a k a* 13 56/64 Joch und 14 Qk, 11) der Wald *V e r h* aber 186 60/64 Joch und 15 Qk. In diesen drei Wäldern wuchsen Birken und Erlen. Die Herrschaft *Frajštanj* behielt sich darinnen nur Eigentumsrechte über die Gründe, also das *Dominium directum*, die Holz-, Weide- und Streurechte hat sie zum Bestand der Dorfgemeinde *Zgornja Polskava* überlassen, wofür einige dortige Untertanen seit jeher in ungeradzähligen Jahren Naturalrobot von drei Pflugtagen und 15 Handrobottagen leisten mußten. Diese Robot wurde mit Einverständnis der Herrschaft wiederruflich mit 4 Gulden 45 kr reluiert.

12) Der Erlenwald von *S t r a ž g o n j c e* maß 206 32/64 Joch. Die vollkommenen Rechte zum Genuß aller Nutzungen aus diesem Wald, wurden vor mehr als hundert Jahren der Dorfgemeinde *Stražgonjce* überlassen, wofür sie jährlich 51 Kapaune, und 5 Gulden 53 kr geben und 153 Tage beim Schnitt helfen mußte. Die Geldleistung wurde rektifiziert und wird in der Bilanz unter Urbarialgiebigkeiten angegeben. Die Giebigkeit der Kapaune wurde mit Einverständnis der Herrschaft reluiert und zwar 12 kr je Stück, die Schnittröbottage wurden ebenso mit 7 kr reluiert, was zusammen 28 Gulden 3 kr einbrachte.

13) Der Buchenwald bei der *M a r i e n k i r c h e* maß 172 12/64 Joch und 6 Qk. Darin hatten die Untertanen von *Podlog* der Herrschaft *Frajštanj* und *Studenice* das unentgeltliche Recht der Viehweide. Die Untertanen der ersten Herrschaft konnten unentgeltlich das Holz und Brennholz beanspruchen, jedoch wurde dieses auf ihr Verlangen von der Herrschaft angewiesen. Das Recht zum Schwenden der Äste mußten jedoch heimische und fremde Untertanen bezahlen.

14) Der Buchenwald bei der Kirche *S v. J a n e z* maß 159 59/64 Joch und 2 Qk. Für diesen Wald galt alles wie für den Wald unter 13).

Es sollte noch angemerkt werden, daß der Untertan fremder Herrschaft *Janez Friedl*, das Recht der Gewinnung des Zaunholzes, aus den Wäldern der Herrschaft *Zgornja Polskava* hatte. Dafür mußte er jährlich zwei Kapaune geben, diese wurden aber für 24 kr reluiert.

Andere Angaben über die Wälder der Herrschaft *Frajštanj* gibt es nicht. Aus obigen Beschreibungen ist aber ersichtlich, daß diese noch weitgehend mit Servitutsrechten belastet und eigentlich in die landwirtschaftliche Sparte gehörten, nur in geringem Umfang waren sie an der Marktwirtschaft beteiligt. Der Verwalter schien sich keine Gedanken zu machen, wie er die Separierung der Wälder zwischen der Herrschaft und den Untertanen zustande bringen könnte, damit sich die erste der leidlichen Servitutsrechte entledigen könnte und in ihren Wäldern rational wirtschaften könnte.

Zusammenfassung

Die Wälder der Herrschaften Žiče und Frajštanj und ihre Bewirtschaftung im Übergang vom 18. ins 19. Jahrhundert

In der Abhandlung werden die Wälder und ihre Bewirtschaftung in zwei Herrschaften, Žiče (Seitz) und Frajštanj (Freystein) beide in der Untersteiermark gelegen, im Übergang vom 18. ins 19. Jahrhundert, beschrieben. Beide Herrschaften gehörten bis zur josephinischen Klösteraufhebung 1782 den Konventen. Žiče dem Kartausenorden daselbst, Frajštanj 1782 aber als auswärtige Herrschaft dem Dominikanerinnenkloster in Studenice. Es wird die Besitzgeschichte beider Herrschaften beschrieben. Nach der Klösteraufhebung gingen beide Herrschaften in den Besitz des Steirischen Religionsfondes über, sie wurden aber durch die Innerösterreichische Staatsgüteradministration in Graz verwaltet. Die erste Herrschaft wurde wegen zu geringer Erträge 1828 an Fürst Werian Windischgrätz, die zweite aus demselben Grund im selben Jahr an Graf Clemens Brandis veräußert.

Vor dem Verkauf wurde für jede Herrschaft sogenannte Gutsbeschreibung verfaßt, worin alle ihre Gründe, Untertanen, Gerechtsame und Verbindlichkeiten beschrieben waren. Auf Grund dieser Beschreibungen werden in der vorliegenden Abhandlung die Wälder beider Herrschaften und ihre Bewirtschaftung gegen Ende des 18. und zu Anfang des 19. Jahrhundert dargestellt. Für die Herrschaft Žiče gibt es in ihrer Historischen Gutsbeschreibung viel Stoff, es wäre aber wegen seiner Allgemeinheit für die behandelte Zeit und Gegend nicht opportun ihn weitschweifig darzustellen. Andererseits sind aber Angaben für die Herrschaft Frajštanj sehr spärlich, deshalb wurde beschlossen beide in einer Abhandlung, jedoch getrennt darzustellen. Das ist insofern berechtigt, da sie ja auch geographisch nicht weit auseinander liegen.

Die Herrschaft Ž i č e hatte 2365 Joch Wälder, in 13 meist kleineren und einigen grösseren Komplexen. Die Wälder waren meistens nicht in besonders guten Zustand, einige wuchsen auf sehr armen, steinreichen Böden in gebirgigen Lagen. Deshalb war die Holzgewinnung im ganzen eher spärlich, jährlich 1 Klafter Eichen-, 40 Klafter Weich- und 343 Klafter Buchenholz. Die Bewirtschaftung der Wälder konnte nicht nach modernen Grundsätzen erfolgen, da sie in grossen Umfang durch Servitutsrechte (zur unentgeltlichen Nutzung des Brenn- und anderweitigen Holzes, der Viehweide, der Streu und der Schweinemast) belastet waren, die die Separierung dieser zwischen der Herrschaft und den Untertanen unmöglich machten. Die räumliche Einteilung der Wälder in Reviere und Abteilungen wurde bis zum Anfang des 19. Jahrhunderts noch nicht durchgeführt. Die Wälder waren auf alte Weise bewirtschaftet. Die Herrschaftsverwaltung wollte wenigstens teilweise ihr Holz geldlich verwerten und plante den Bach Žičnica zur Trift des Holzes auszubauen und zwei Holzplätze in Konjice und Loče anzulegen. Diese Verwaltung hatte ausnehmend grosses Verständnis für die Nöte der Untertanen, die die Mitnützung der Wälder für ihre eher zurückgebliebene Wirtschaftsweise dringend benötigten. Sie hatten alteingesessene Rechte, z.B. der freien Auswahl der Bäume zum Fällen in den herrschaftlichen Wäldern, die sie sich keinesfalls schmälern liessen. Die Separierung der Nutzungsrechte wurde in der Zeit der Verwaltung durch die Innerösterreichische Staatsgüteradministration bis 1828 nicht in Angriff genommen, erst in den sechziger Jahren des 19. Jahrhunderts unternahmen dies die Förster des Fürsten Windischgrätz.

Ihr Bestreben wurde aber durch eine Gerichtsentscheidung zugunsten der Bauern zurückgewiesen. In dem Abschnitt über die Herrschaft Žiče werden eingehend die Gründe für die Separierung (Abtretung) eines Teils der herrschaftlichen Wälder an die Untertanen erörtert. Diese Separierung gelang aber erst in den letzten Jahrzehnten des 19. Jahrhunderts.

Geldliche Einnahmen hatte die Herrschaft Žiče von Wäldern nur aus zwei Nutzungen, aus dem Holzverkauf und der Schweineweide, die natürlich vom Fruchten der Büchelkerne und der Eicheln abhängig war. Aus der ersten Nutzung wurden im 9-jährigen Durchschnitt 87 Gulden, aus der zweiten durchschnittlich 36 Gulden eingenommen. In den Jahren 1794 und 1797 beliefen sich die Einnahmen aus der Schweineweide auf 113 bzw. 166 Gulden, vom Holz jedoch auf 148 bzw. 36 Gulden. Wenn man die Einnahmen aus den Waldnutzungen und Ausgaben für den Gehalt des Holzbedienten und sein Holzdeputat vergleicht, ersieht man, dass die Wälder in dem behandelten Zeitraum nicht rentabel waren.

Die Herrschaft Frajštanj hatte 14 kleinere und grössere Wälder mit der Gesamtfläche von 1423 Joch. Die meisten Wälder lagen in ziemlich sumpfiger Ebene und wurden hauptsächlich mit Erlen, Birken und wenig mit Eichen bewachsen. Nur in den angrenzenden Hügeln waren einige Buchenwälder. Fast alle Wälder waren mit Servitutsrechten für Behölzung, Viehweide und Streu behaftet. Diese Rechte standen den benachbarten Dorfgemeinden zu. Bei dieser Herrschaft konnte eine Besonderheit ermittelt werden und zwar das Miteigentum mehrerer Herrschaften und einer Dorfgemeinde an einigen Wäldern. So teilten sich an einigen Wäldern das Eigentumsrecht gleichberechtigt die Herrschaften Frajštanj, Rače, Zgornja Polskava, das Gut Pragersko sowie die Gemeinde Nova vas. Das Holz, meistens von Erlen, wurde im Spätherbst oder zu früher Winterzeit hauptsächlich für die Bedürfnisse der Herrschaften geschlagen. Ein milderer Teil wurde geschlagen, von unparteiischen Männern geschätzt und in der Lizitation den Meistbietenden zugeschlagen. Auf dieser Herrschaft war moderne Waldbewirtschaftung nicht einmal in Ansätzen vorhanden. Zur behandelten Zeit gab es auch keine Ansätze zur Separierung der Wälder zwischen der Herrschaft und den Untertanen um die leidliche Servitutsfrage irgendwie zu lösen. Auf dieser Herrschaft gibt es keine Angaben über Einkommen und Ausgaben, jedoch ist es sicher, dass von der Waldnutzung keine erheblichen Einnahmen, aber auch keine Ausgaben herrührten, da die Herrschaft keinen Holzknecht hatte und keine Waldpflagemassnahmen ausführte.

Povzetek

Gozdovi gospostev Žiče in Frajštanj in gospodarjenje z njimi na prehodu iz 18. v 19. stoletje

V razpravi so prikazani gozdovi in gospodarjenje z njimi na dveh spodnještajerskih gospostvih Žiče in Frajštanj na prehodu iz 18. v 19. stoletje. Obe gospostvi sta do jožefinske odprave samostanov 1782 pripadali konventom, prvo kartuzijanskemu redu prav tam, Frajštanj pa kot zunanje gospostvo samostanu dominikank v Studenicah. Opisana je posestna zgodovina obeh gospostev. Po ukinitvi samostanov sta obe gospostvi prešli v last in posest Štajerskega verskega sklada, upravljala pa jih je Notranjeavstrijska uprava državnih posestev v Gradcu. Zaradi prenizkih donosov sta

bili obe gospostvi 1828 prodani. Žiče knezu Weriandu Windischgrätzu, Frajštanj pa grofu Clemensu Brandisu.

Pred nameravano prodajo so za vsako gospostvo sestavili tako imenovani Opis posestva, v katerem so bila zajeta vsa zemljišča, podložniki, vse gospostvene pravice in obveznosti. Na podlagi teh opisov so v pričujoči razpravi prikazani gozdovi in gospodarjenje z njimi na obeh gospostvih na prehodu iz 18. v 19. stoletje. Za gospostvo Žiče je v tem zgodovinskem opisu veliko snovi. Ne bi pa bilo smiselno, da bi to tvarino zaradi splošnosti za obravnavani čas in za tamkajšnjo okolico obširno obravnavali. Po drugi strani pa so podatki za gospostvo Frajštanj prav skromni. Zato smo se odločili, da obe gospostvi predstavimo v skupni razpravi, vendar ločeno. To je upravičeno toliko bolj, ker tudi zemljepisno nista daleč narazen.

Gospostvo Žiče je imelo 2365 oralov gozdov, v 13, večinoma majhnih in v nekaj večjih kompleksih. Gozdovi večinoma niso bili v posebej dobrem stanju, nekaj jih je bilo na zelo nerodovitnih tleh in v hribovitih, strmih legah. Zato je bila sečnja lesa sorazmerno skromna, letno 1 klafter hrastovega, 40 klafter lesa iglavcev in 343 klafter bukovega lesa. Gospodarjenje v gozdovih ni bilo mogoče po sodobnih načelih, ker so bili še v velikem obsegu obremenjeni s služnostnimi (servitutnimi) pravicami v korist podložnikov za brezplačno pridobivanje drv in drugega potrebnega lesa, za pašo živine, zlasti tudi svinj, in tudi za pridobivanje stelje. To je onemogočalo, da bi izvedli oddelitev (separacijo) gozdov med gospostvom in podložniki. Prostorska razdelitev gozdov v revirje in oddelke do začetka 19. stoletja še ni bila opravljena. Z gozdovi so gospodarili na star način. Uprava gospostva je želela vsaj delno doseči za svoj les denarno prodajo in je zato načrtovala, da bi potok Žičnico uredila za plavljenje in da bi si uredila dve lesni skladišči v Konjicah in Ločah. Ta uprava je imela izjemno veliko razumevanje za težave podložnikov, ki so souporabo gozdov nujno potrebovali za njihovo bolj ko ne zaostalo gospodarjenje na njihovih posestvih. Ti so imeli stare od nekdanj uveljavljene pravice, npr. proste izbire drevja za posek, ki si jih nikakor niso pustili kratiti. Separacijo gozdov za odpravo služnostnih pravic v času upravljanja prek Administracije državnih posestev v Gradcu do 1828 še niti niso začeli. Šele v šetsdesetih letih 19. stoletja so poskušali gozdarji kneza Werianda Windischgräetza kratiti pravice podložnikov. Njihov poskus pa je bil s sodno odločitvijo v prid kmetov zavrnjen. V poglavju o gospostvu Žiče v tej razpravi so podrobno prikazani razlogi za separacijo enega dela gospostvenih gozdov podložnikom za odstop njihovih služnostnih pravic. Ta separacija pa je uspela šele v zadnjih desetletjih 19. stoletja.

Denarne dohodke je imelo gospostvo Žiče od gozdov le iz dveh virov, iz prodaje lesa in dajatev za svinjsko pašo, ki je seveda bila odvisna od rodnosti hrastov in bukev za želod oz. žir. Iz prvega vira so bili dohodki (po devetletnem povprečju) 87, iz drugega vira pa v povprečju 36 goldinarjev. V letih 1794 in 1797 pa so bili dohodki od dajatev za svinjsko pašo 113 oz. 166, od lesa pa 148 oz. 36 goldinarjev. Če seštejemo dohodke od gozdov in od njih odštejemo plačo za gozdnega hlapca, 100 goldinarjev ter še lesni deputat, je več kot razvidno, da žički gozdovi v obravnavanem času sploh še niso bili rentabilni.

Gospostvo Frajštanj je imelo 14 večjih in manjših gozdov v skupni izmeri 1423 oralov. Večina gozdov je ležala v sorazmerno močvirni ravnini v okolici Zgornje in

Spodnje Polskave in so bili zaraščeni večinoma z jelšami, brezami in nekoliko s hrasti. Le na obronkih bližnjih hribov je bilo nekaj bukovih gozdov. Skoraj vsi gozdovi so bili obremenjeni s služnostnimi pravicami podložnikov za pridobivanje lesa, za pašo živine in za pridobivanje stelje. Te pravice so uživale sosednje občine. Pri tem gospostvu smo ugotovili posebnost in sicer enakopravno solastnino več gospostev in ene vaške občine na nekaterih gozdovih. Tako so imela solastnino na nekaterih gozdovih gospostva Frajštajn, Rače, Zgornja Polskava in posestvo Pragersko ter vaška občina Nova vas. Drevje v teh gozdovih so podirali v pozni jeseni ali v zgodnji zimi, večinoma za potrebe gospostev. Manjši del so posekali, ga dali oceniti nepristranskim cenilcem in so ga na dražbi prodali najboljšim ponudnikom.

Na tem gospostvu ni bilo niti sledov o modernem gospodarjenju z gozdovi. V obravnavanem času niti ni bilo zamisli o separaciji gozdov med podložniki in gospostvom, da bi na nek način rešili težavno vprašanje služnostnih pravic. Na tem gospostvu ni nikakršnih podatkov o dohodkih in izdatkih v zvezi z gozdovi, vendar je zanesljivo, da ni bilo omembe vrednih dohodkov, pa tudi nikakršnih izdatkov, ker gospostvo ni imelo gozdnega hlapca in ni izvajalo nikakršnih gozdnogojitvenih ukrepov.

Agrovoc descriptors: farmland; nitrates; leaching; soil transport processes; groundwater; water resources; groundwater pollution; soil pollution; precipitation; irrigation; fertilizers; intensive farming

Agris category codes: P10, P01, T01

COBBIS code 1.02

Causes of nitrate leaching from agriculture land in Slovenia

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ABSTRACT

In Slovenia, groundwater is significant source of drinking water. It has been widely reported that contamination of groundwater from agricultural non-point source is one of the major pollution problems. The pollution of groundwater in areas of agricultural activity is a result of using of mineral nitrogenous fertilizers in large quantities. Applying excess nitrate fertilizers directly affects ground water quality, especial for $\text{NO}_3\text{-N}$, which is highly mobile. Non-point loss of $\text{NO}_3\text{-N}$ from fields to water resources however is not caused by one single factor. Rather is caused by combination of factors including precipitation, crop uptake, irrigation, application of fertilizers and soil property. Although Slovenian areas of intensive agriculture have different soil characteristics, different rainfall regime and agricultural practice, their nitrate leaching regime appears to be similar and in majority related to the precipitation and fertilization.

Key words: nitrate leaching, precipitation, irrigation, fertilizers, water pollution

IZVLEČEK

VZROKI IZPIRANJA NITRATA IZ KMETIJSKIH POVRŠIN V SLOVENIJI

Podtalnica je v Sloveniji pomemben vir pitne vode. Znano je, da intenzivno kmetijstvo kot netočkovni vir obremenjevanja, predstavlja glavni problem onesnaževanja podzemnih voda. Na kmetijskih zemljiščih z intenzivno pridelavo prihaja do prekomerne porabe mineralnih gnojil, kar negativno vpliva na kakovost podtalnice. Uporaba dušičnih gnojil v količinah, ki presegajo zahteve posevkov, poveča izpiranje dušika v obliki zelo topnega in mobilnega nitrata v globlje plasti tal in podtalnico. V kolikšnem obsegu bo nitrat iz površinskih slojev obdelovalnih tal prodril v podtalje, ni odvisno samo od količine dušičnih gnojil, ampak tudi od količine padavin, vrste vegetacije, namakanja ter lastnosti tal. Kljub temu, da se v Sloveniji intenzivna kmetijska območja razlikujejo po količini padavin, kmetijski praksi ter talnih lastnostih, je dinamika izpiranja nitrata na vseh območjih predvsem odvisna od vremenskih razmer in gnojenja.

Ključne besede: izpiranje nitrata, padavine, namakanje, gnojila, onesnaževanje voda

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1 INTRODUCTION

Intensification of agricultural activities, such as fertilization practice and application of irrigation has dramatically increased crop yields. Like any enhanced productive activity it leads to environmental contamination if it is improperly managed (Hadas et al., 1999). Excessive use of N fertilizers and irrigation increase the risk of percolation and leaching of nitrates to groundwater (Bijay-Singh et al., 1995).

Nitrogen is a widely used plant nutrient, which is essential for the growth and development of a healthy crop. It is most often the limiting nutrient in plant growth and to overcome this limitation, N fertilizers are used to increase crop yields (Jalali and Rowell, 2003). The high inputs of N fertilizers had markedly modified the N cycle. The N cycle include several nitrogen transforming processes such as mineralization, immobilization, nitrification and denitrification which are very complex and dynamic. The nitrification process corresponds to the transformation of the manure's nitrogen into nitrate. Nitrification is a biological process during which nitrifying bacteria (*Nitrosomonas* and *Nitrobacter*) convert ammonium to nitrate. The nitrate form of nitrogen is the form most available to plant. But, this form is very soluble in water and therefore, very mobile within soil solution.

Application of nitrogen fertilizer in excess can lead to significant nitrate leaching out of the root zone, because plant uptake and microbial immobilization can not remove the entire nitrate from the solution (Pratt and Adriano, 1973). Below this layer, nitrate is not actively absorbed by roots and has high potential to move downwards. So the amount of nitrate lost is depended on the quantity of nitrate available to be leached. Another factors affecting nitrate leaching are the precipitation-intense rainfall events or surplus of water provided by irrigation, evapotranspiration, drainage, soil texture, soil porosity and occurrence of preferential flow paths (Wu et al., 1997; Cameria et al., 2003).

Nitrogen losses not only result in lower production and subsequent increase in production costs but can also lead to groundwater deterioration and pollution (Cepuder and Shukla, 2002). Contamination can render groundwater unsuitable for human consumption. Nitrate frequently pollutes groundwater supplies (Spalding and Exner, 1993). The high concentration of nitrate in water supplies can cause ecological damage and health hazards. Several studies document adverse effects of high nitrate levels most notably methemoglobinemia and stomach cancer (Ward et al., 1994). Nitrate also causes algal blooms that can produce toxins and lower the oxygen content of waters, both of which adversely affect aquatic organisms. This eutrophication is a widespread problem that is persistent and slow to recover.

The groundwater nitrate problem is international scope. Nitrate concentration exceeding the international (WHO, 1993) recommendations for drinking water (50 mg NO₃⁻/l) have been found in groundwater under 22% of cultivated land identified nitrate and ammonium in a sandstone aquifer beneath Nottingham, England (Rivers et al., 1996).

In Slovenia, groundwater is major source of drinking water. Because of the risk of agricultural impact on groundwater contamination, also in Slovenia, the nitrate

leaching received considerable attention. The aims of this paper is reviewed and discuss about the most important factors affecting nitrate leaching from agriculture land and estimate how combination of factors including precipitation, crop uptake, irrigation, application of fertilizers and soil property affect the dynamics of nitrate leaching in Slovenia.

2 MATERIALS AND METHODS

This article is written in the form of a literature review of recent scientific publications related to nitrate leaching from agricultural land in Slovenia. The review is not just a summary of the resources but it is also evaluation and shows relationships between different studies.

3 RESULTS AND DISCUSSION

3.1 The effects of precipitation on nitrate leaching

Rainfall is one of the most important factors affecting the movement of nitrate in agricultural soil. Results of study conducted in Apače valley (average annual precipitation 939 mm) in the period between March (1993) and December (1995) confirm this contention and show that groundwater nitrate pollution was more pronounced in years with higher precipitation (1994 – max N-NO₃ in groundwater was 71.1 mg /l in 1995 – max was 58.6 mg N-NO₃/l) than in year with lower precipitation (1993 – max N-NO₃ in groundwater was 20.5 mg/l) (Pintar et al., 1996).

Rainfall that infiltrates the soil surface may causes nitrate ions to move down through the soil profile by percolation. The more rain that falls and infiltrates in the period after application of N fertilizer, further down in the profile nitrate ions move (Bugar, 1999). If precipitation exceeds evapotranspiration, nitrate can leach to a groundwater. But where the amounts of rainfall are low and potential evapotranspiration exceeds annual precipitation, the concentration of nitrate tends to be high because the diluting effect is reduced (Leskošek, 1994).

In northeastern part of Slovenia (Prekmurje) average precipitation are low and evapotranspiration is relatively intensive, so small quantities of water percolates through the soil profile. Consequently, because of less dilution, there is a higher concentration of nitrates in the groundwater (Leskošek, 1994). In central and western part of country percolation of water through the soil profile is comparatively higher. Owing to the large volume of water flowing through, there is a dilution of nitrate content, and the probability of groundwater pollution with nitrate is consequently smaller (Action programme..., 2004).

Slovenian's climate is generally characterized by heavy precipitation (800-3500 mm/year), and there is a very high risk of nitrogen leaching even during the growing season (Leskošek, 1994). Leaching is especially intensive in autumn and through the winter, when there is no active uptake of N into plants, while at the same time evapotranspiration is low, so the water surplus is at its highest. The other period,

when the danger of leaching arises in Slovenia is May and the first half of June when there is a maximum precipitation (Action programme..., 2004).

Also the study in the Slovenian coast region with submediterranean climate (900 mm) confirms that precipitation regime could significantly influence on nitrate leaching. The results show that excessive rainfall causes nitrate movements downward below the root zone, because after the heavy rain nitrate concentrations in drainage water significantly decreased (Figure 1). The highest concentration of nitrate was found during spring and autumn months. In the Slovenian coast region most of the annual precipitation falls in these months (Podgornik, 2003). The maximum nitrate concentrations (94.4 mg N-NO₃/l) in drainage water were observed in the end of growing season. Soil water nitrate concentration is the highest at the onset of the autumn rainfall and progressively decrease as nitrate is flushed out of the soil (Podgornik, 2003).

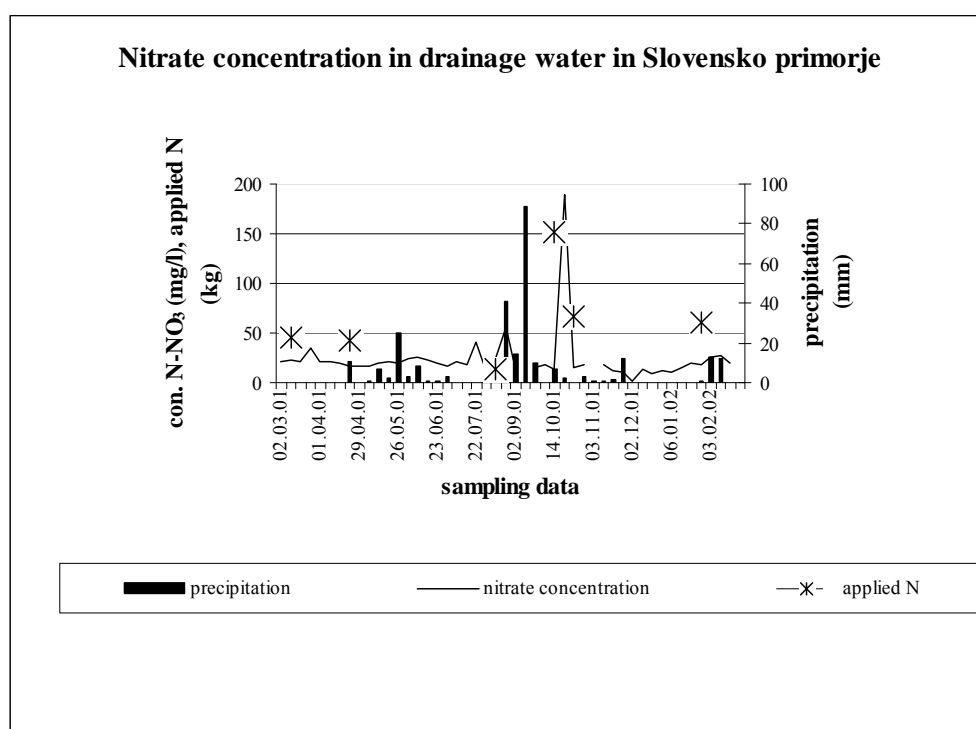


Figure 1: Impact of applied fertilizer (kg) and precipitation (mm) on nitrate concentration (mg/l) in drainage water Zontarji (in Slovensko primorje region - W Slovenia)

From data of average yearly precipitation, average yearly potential evapotranspiration, and effective field capacity, the approximation of average yearly potentially leached water below the root zone was calculated. This was used to determine zones with potential risk of soil percolate contamination with nitrate in Slovenia. The nitrate vulnerable zones are predominantly in the north – east part of Slovenia. However because of relatively high amount of potentially leached water below the root zone there would be no risk of leachable pollution with nitrate on the 86% of Slovene territory (Mihelič et al., 2002).

3.2 Influence of some soil properties on nitrate leaching

Karst with limestone as a geological ground, occupies around 44% of the Slovenian territory. This region wasn't be identified as vulnerable for nitrogen leaching into ground water, because has limiting growing condition for agriculture crops (i.e. climate, soil depth) and clay soil with high water holding capacity. The average yields and uptake by crops are low, consequently fertilizer application rate is low and therefore non – point source pollution caused by mineral fertilization is not considered as a serious problem in this region. The high nitrogen surpluses can be caused by high animal density per hectare. In this case, manure can be considered as a serious pollution source (Matičič, 1998).

Nitrate leaching is strongly affected by the particle size distribution, the soil porosity, and the occurrence of preferential flow paths (Cameria et al., 2003). Soils have varied retentive properties depending on their texture and organic matter content. Soil texture refers to the relative proportion of particles of various sizes in a given soil (Gaines and Gaines, 1994). Due to the higher proportion of gravitational pores coarse soils are usually more vulnerable to leaching than clayey soils (Wu et al. 1997). They observed that up to 50% of applied nitrogen can be leached from coarse soils. Sandy soils are also fairly homogeneous, water moves freely through much of the soil matrix. Nitrate that is in the soil, whether from fertilizer or from microbial activity, is likely to be carried through the soil slowly but surely with little impediment (Addiscott, 1996).

Nitrogen loss to a groundwater from clay soil is smaller than those from the coarse-textured soils. Finer clay soils have more retention capacity than sandy soil, because sandy soil have less silt and clay which gives rise to lower cation exchange capacity (Gaines and Gaines, 1994).

The negative charge on the clay particles retains ammonium ions (NH_4^+). Retention of ammonium ions on clay particles protects them (ammonium ions) from leaching. Nitrate ions (NO_3^-) are negatively charged and are not retained by clay particles (Leskošek, 1993). Clay soil does not specifically retain nitrates, but water does not pass easily through clay soil. Large surface areas of the individual clay particles and the large number of very small pore spaces can hold a large amount of water. Water-filled pores of clay soils lack oxygen. Lacking oxygen, a group of soil bacteria, called facultative anaerobes, substitute nitrates for oxygen for respiration. When bacteria use nitrates as a substitute for oxygen, they convert nitrates to nitrogen gas through a process called denitrification. Nitrate loss through denitrification in clay soils reduces the amount of nitrates that can be potentially leached to groundwater (Bhumbla, 2006).

But fine textured soils with frequent worm holes, cracks, or other vertical channels have the potential to allow nitrate movement deep into the soil beyond the rooting zone. In this circumstance, significant amounts of water may flow through large soil

pores (macro pores) even through they make up only small percentage of total pores. This type of water flow is called preferential (Addiscott, 1996).

Soil thickness and distance between the root zone and groundwater also determine the vulnerability of an aquifer to pollution. Nitrate leaching from shallow soils on fractured soils as limestone can cause extensive contamination of groundwater. There are numerous reports of nitrate movement to ground water from agricultural soil in karst regions (Bhumbla, 2006).

3.3 Impact of irrigation on the dynamics of nitrate movement through a soil profile

The dynamics of nitrate leaching for four different advanced irrigation and fertilization practices was studied in Savinja valley – east part of Slovenia. The field experiment was carried out in intensive hop garden, with continental climate (790 mm precipitation per year). The first one - control consist of non-irrigated and surface fertilized hop with 217 kg N/ha in three consecutive rations. The second and the third practices comprise sprinkler and classical - drip irrigated hop, making use of the same fertilization as the first one. The fourth practice is fertigated hop, where amount of added nitrogen was 220 kg N/ha on soil surface (Pintar, 1999).

No clear differences were observed among the variations of non irrigated, sprinkler irrigated and drip irrigated hop (Fig 2, Fig 3, Fig 4). Analysis of results shows that the greater part of collected samples of percolated soil water from fields under non irrigated, sprinkler irrigated and drip irrigated hop had nitrate concentration over 50 mg NO₃⁻/l.

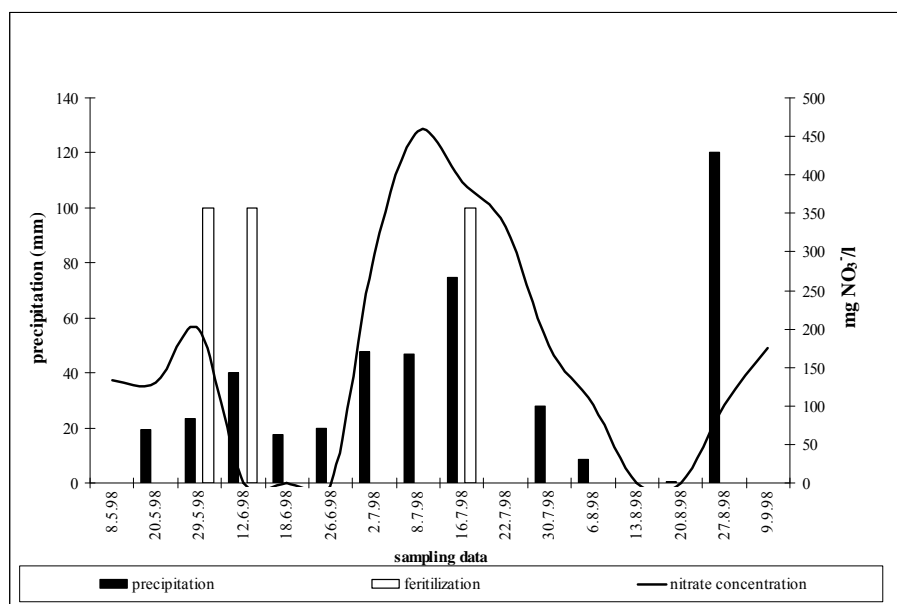


Figure 2: Impact of fertilizer application (kg) and precipitation (mm) on nitrate concentration (mg NO₃⁻/l) in percolating soil water in control variant in hop garden in Savinja Valley

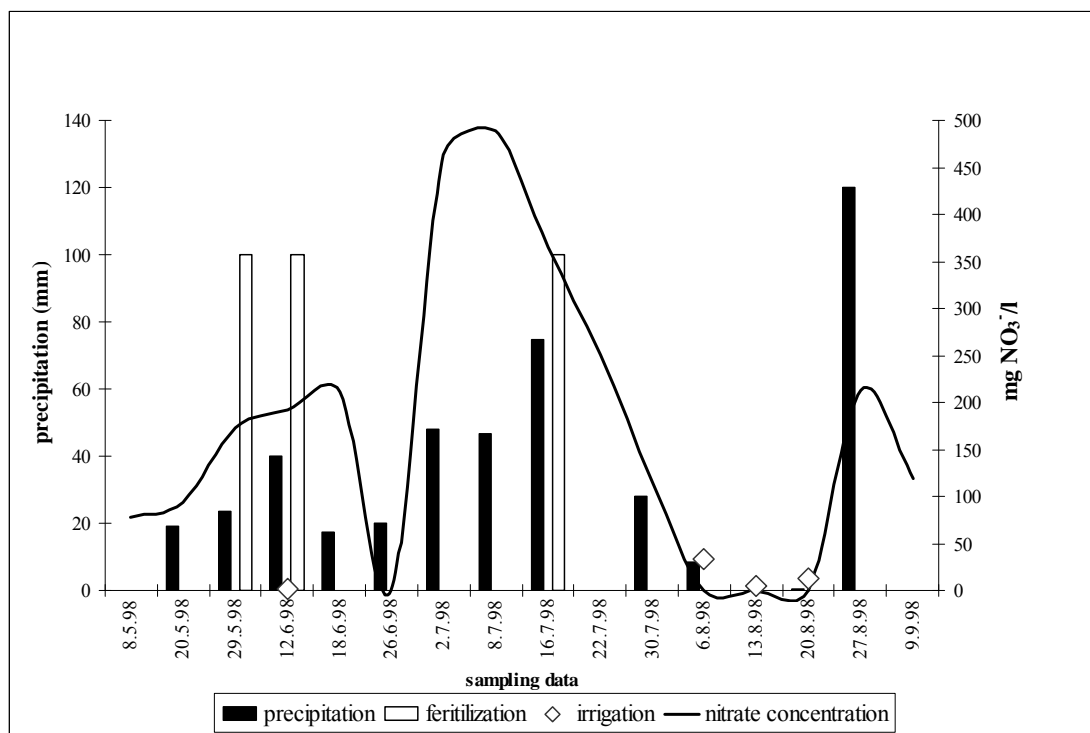


Figure 3: Impact of fertilizer application (kg) and precipitation (mm) on nitrate concentration (mg NO₃⁻/l) in percolating soil water in sprinkler irrigated variant in hop garden in Savinja Valley

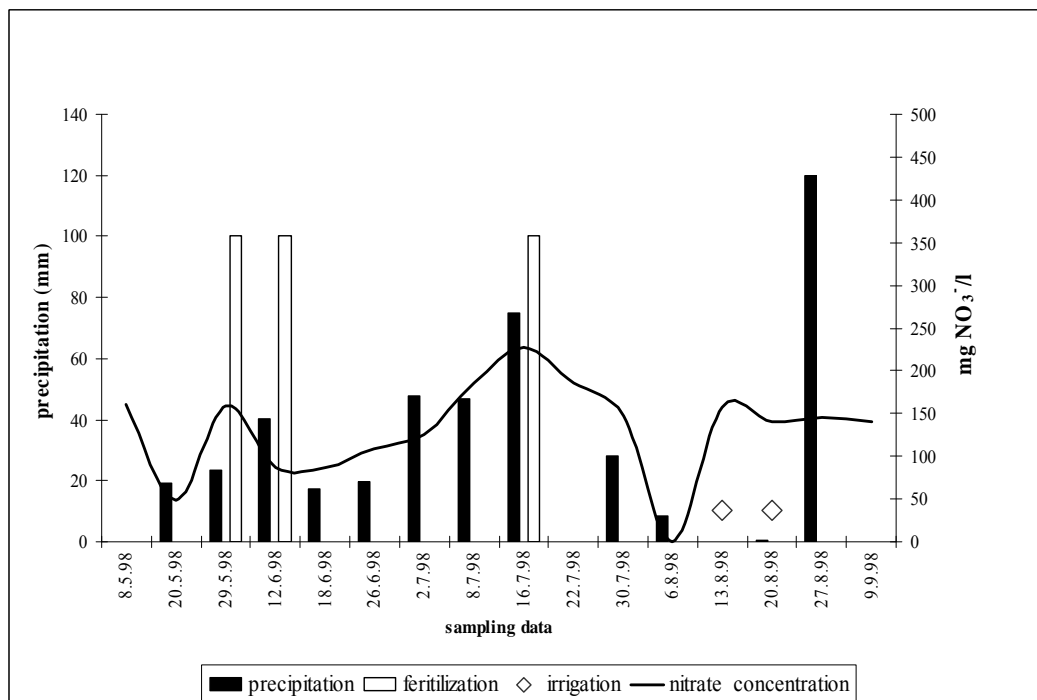


Figure 4: Impact of fertilizer application (kg) and precipitation (mm) on nitrate concentration (mg NO₃⁻/l) in percolating soil water in classical – drip irrigated variant in hop garden in Savinja Valley

After rainfall events and application of fertilizer in all treatments with classic fertilizing practices significant increase of nitrate concentration in percolated soil water was observed. Present study indicated that during the rainfall period, nitrate leaching was lower in drip irrigated hop (226.70 mg NO₃⁻/l) treatment than in non-irrigated treatment (457.3 mg NO₃⁻/l) and sprinkle irrigated hop (488.60 mg NO₃⁻/l).

The significant difference in nitrate leaching was observed only in the treatment involving fertigation (Fig 5). In fertigation treatment in percolating soil water accumulated nitrate concentration was lower than in the control variant.

The large amount of water used in agriculture makes the risk of leaching nitrates and other chemicals potentially greater in areas that are irrigated. The increase in soil moisture that results from irrigation dissolves excess nitrate present in the soil profile and makes it more susceptible to leaching (Casey et al., 2002). Higher moisture contents will also raise microbial activity including mineralization (Skopp et al., 1990). The increase in mineralization rates has been shown to directly affect nutrient leaching (Doran, 1980).

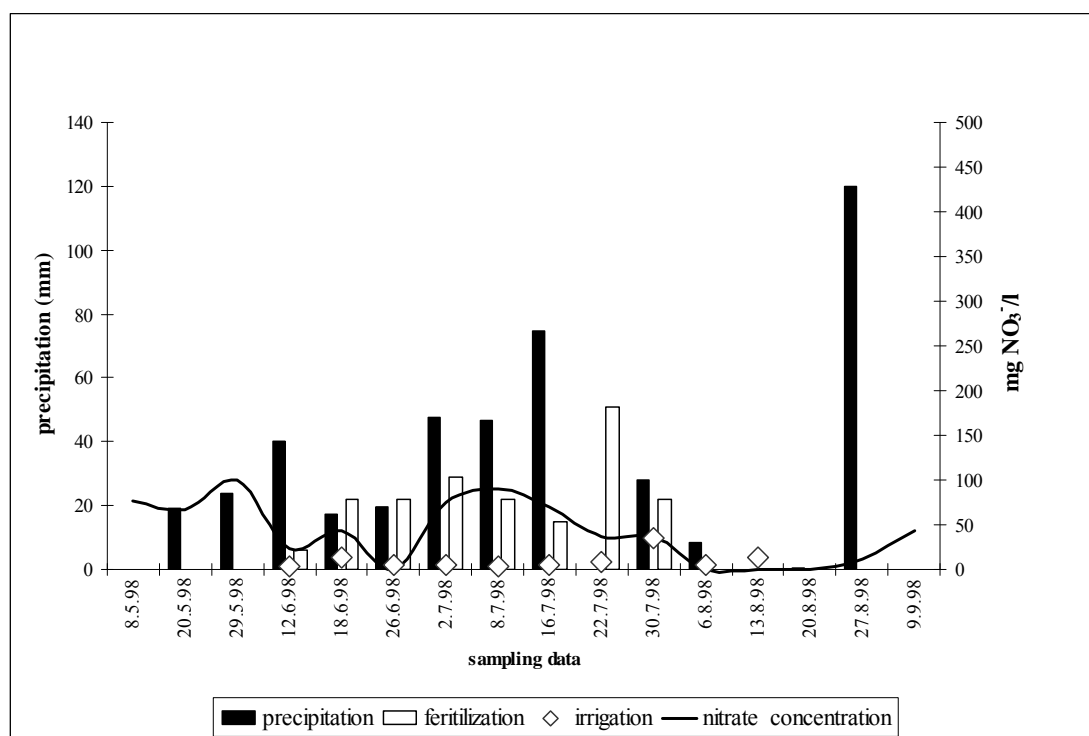


Figure 5: Impact of fertilizer application (kg) and precipitation (mm) on nitrate concentration (mg NO₃⁻/l) in percolating soil water in fertigation in hop garden in Savinja Valley

Also the method of irrigation of water application influences the leaching process. Most of the irrigation in the world is surface irrigation. The efficiency of water use in such system is low and one half to one third of applied water may be lost carrying considerable amounts of nutrients with it. But there are also advanced irrigation

systems available, like sprinkler and drip irrigation. Sprinkler irrigation system has a higher efficiency and water nutrient losses may be hugely controlled. Drip irrigation system can be used for fertilizer application that is known as "fertigation". Fertigation or the use of irrigation system to apply fertilizers to crop, has gained popularity in recent years, because it was demonstrated that it is possible to use N fertigation for high yield with minimum nitrate leaching (Mohammad et al., 2002). As indicated by other authors (Mohammad et al., 2002), this irrigation method restricts deep percolation losses and can be most effective for prevention of nitrate losses.

However irrigation appears to be also the most important factor in reducing potential for nitrate leaching. Schedule irrigation appropriately by monitoring soil water content and crop water use and right time water applications reduce nitrate leaching from irrigated fields (Seelig and Nowatzki, 2001).

3.4 Fertilizers and nitrate leaching

The results of study in Mediterranean part of Slovenia clearly demonstrated that fertilizer application rate have a significant influence on the amount of nitrate leached out of the root zone. On the experimental field, amount of applied N fertilizer was less than is recommended by technology. Fertilizer application was done in autumn or spring. The greatest concentration of nitrate was found in drainage water after the fertilizer application (Figure 1). The highest nitrate concentration of the study period was 94.4 mg N-NO₃/l found in drainage in the end of October. The lowest nitrate concentration (1.09 mg N-NO₃/l) was measured in December. Ninety-two percent of samples had nitrate concentration higher than the limit value for good groundwater chemical status (50 mg NO₃⁻/l) (Podgornik, 2003).

Study in the early 1900s showed that many farmers planted legumes in their crop rotation to replenish soil nitrogen (Bundy et al., 1994). But after the Second World War application and availability of mineral nitrogenous fertilizers increased and demand for forage crops led to a significant reduction in crop rotations and a general substitution of purchased nitrogen for biological nitrogen (Dinnes et al., 2002). Since that time nitrogen fertilizer use has increased rapidly. One reason for the increase has been fertilizer nitrogen's low price relative to the value of the crop (Mihelič, 1997). These changes increased the amount of nitrate leached from agricultural soil.

Nitrate leaching from fertilizer use depends upon the fertilizer types (ammonium, nitrate or organic), method of application, and climate condition. Nitrate leaching may be greater when fertilizer contains the nitrate compared to the situations where ammonium nitrogen is the major component of a fertilizer (Bhumbla, 2006). Animal manure N provides many physical, chemical and biological benefits to soil, but on the other side, in mineral fertilizers nutrients are more readily available for plant uptake.

Either the N source is animal manure or commercial N fertilizer, over-application or ill-timed-application, either source can provide too much plant-available N and increase the potential for nitrate leaching (Addiscott, 1996). Nitrate losses are likely

to be higher when all nitrogen is applied in one application compared to when nitrogen is applied in split applications. Nitrate losses from fertilizer use can be reduced by matching fertilizer application with nitrogen needs of a crop (Bhumbla, 2006).

Slovenian national statistical data show that fertilizer and nitrogen consumption has slightly decreased since the year 1998 (Globevnik et al., 2006), but in large part of Slovenia, farmers still use excessive amount of fertilizer, chiefly in the lowland plains, which offer the highest intensity of agricultural production. This confirms also a balance of nitrogen, which in other words means excessive use of fertilizer, calculated for individual areas as a weighted average for second level hydrogeographical areas in Slovenia. The highest surplus, (more than 120 kg N/ha per year) appears toward the east of Slovenia - lowland plains, meanwhile the lowest surplus of nitrogen (less than 80 kg N/ha per year) appears toward the west part of Slovenian hilly area (Globevnik et al., 2006).

3.5 Agricultural crops affect nitrate leaching

Due to significant difference between natural and agricultural ecosystems, the object of study in central part of Slovenia on Ljubljana filed (average annual rainfall 1400 mm) was to quantify the nitrate leaching from natural land never has been ploughed or fertilized (Karahodžič, 2006; Gvardjančič, 2006).

Percolated soil water has been sampled in a lysimeter on a depth of 2.5 m below soil surface. In a study period 2002-2005 the majority of nitrate leaching was related to several precipitation events. Nitrate concentration in percolating water showed variations among years (Figure 6).

During the first year of the study (2002), the maximum nitrate concentration in percolated water was very low (8.18 mg NO_3^-/l). In October 2003 nitrate concentration in percolating water drastically increased (33.66 mg NO_3^-/l). The highest nitrate concentration (37.80 mg NO_3^-/l) of the study was observed during the spring 2005. Furthermore samples collected in April 2005 exceeded also the recommendation for good groundwater chemical status (25 mg NO_3^-/l). Reasons may be addressed to the atmospheric N input.

In order to increase the yield of agricultural crops, addition of N fertilizer to agricultural ecosystem has markedly modified the N cycle (Sprenst, 1987). Beside a disturbance of N cycle, also harvest is the point when arable land becomes most different from natural system. The soil is bare and there are no plants to take nitrate from the soil and balance between rainfall and evaporation is changing (Addiscott, 1996). The quantity of N removed during harvest is generally 30 to 70 percent of the total quantity of N applied as fertilizer (Hermanson et al., 2000). Nitrogen, not removed with the harvest, either remains as NO_3^- in the soil, in the remaining plant residue, or is lost to the atmosphere as either ammonia (NH_3) or elemental nitrogen (N_2) and nitrous oxide (N_2O) after denitrification.

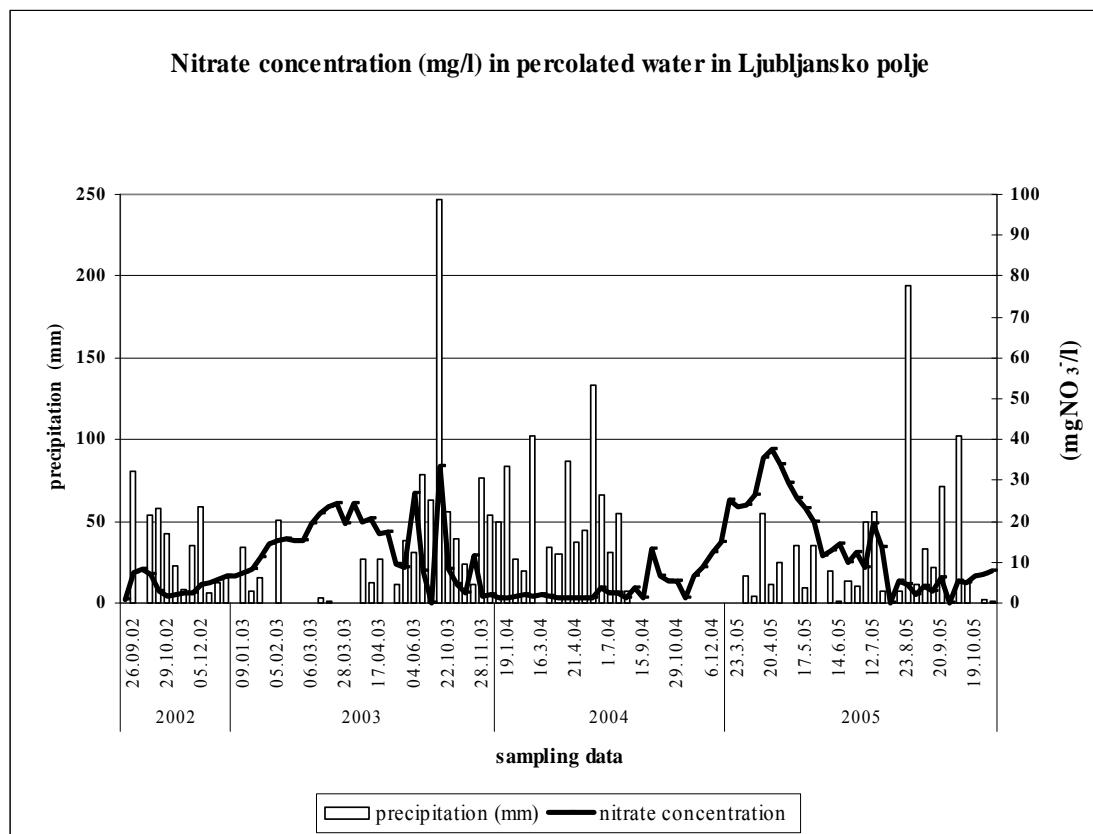


Figure 6: Nitrate nitrogen concentration (mg NO₃⁻/l) in percolating water under never fertilized soil in Ljubljana field.

Due to NH₃ from agriculture activities and emission of NO_x from combustion process, the atmospheric N input to natural system in Europe and North America has increase dramatically during the last decades. Natural systems of vegetation usually have small concentrations of nitrate in the water draining from soil (Addiscott, 1996). Forest ecosystem may accumulate considerable amounts of N in biomass and soil organic matter, but there is an increasing concern that forest ecosystems may be overload with N from atmospheric deposition. Indeed, increased leaching of nitrate has been observed in several areas of high N deposition (Gundersen and Bashkin, 1994).

The great diversity exists also within and between nitrogen cycles of natural ecosystems (Gundersen and Bashkin, 1994). According to data nitrate leaching in natural coniferous forests is less than 1kg N/ha/year (Hauhs and Wright, 1986), somewhat higher in deciduous forests, but still less than 2-3 kg N/ha/year. The amount of nitrate-N leached from natural background in one year (never ploughed or fertilized) in Ljubljana differed between 9 kg/ha (2003) and 15 kg/ha (2004).

4 CONCLUSIONS

Comparison of nitrate concentration in soil water of different agricultural practices and nitrate concentration in percolating soil water of natural background indicates that change from natural habitat to intensive agricultural practice leads to increased nitrate losses and pollution risks. Nitrogen fertilizers can supply large quantities of N to arable soils but also increase the amount of nitrate at risk to leaching.

In arable cropping systems with annual crops, receiving N applications in excess of agronomic rates, leaching of nitrate during winter is difficult to avoid under many conditions where precipitation are available during the non-growing season. Soils may contain substantial amounts of nitrate during this period, but crop uptake is small and rainfall exceeds evaporation. During this period the greatest quantities of nitrate are leached from arable soils.

Although Slovenian areas of intensive agricultural have different soil characteristics, different rainfall regime and agricultural practice their nitrate leaching regime appears to be similar and related in majority to the precipitation and fertilization.

Fertigation is a good way to minimise the leaching of nitrate. Improved crop water supply during dry periods also enhances crop production and often increases nitrogen uptake from the soil. Not only scheduling of irrigation, but also split application of N fertilizer and proper time of application is necessary for good N management practices that will reduce nitrate leaching.

Cover crops that would take up mineral N and reduce soil nitrate, accumulated by the beginning of spring, can also reduce the risk of nitrate leaching. The high soil nitrate accumulation during autumn and winter can be reduced by growing winter cover crops which take up N during the wet season when leaching is likely.

But nitrates, moved downward by leaching, can come from many sources, not necessarily just from fertilizers. Natural source includes nitrate incorporated in rock or nitrate from atmospheric deposition. So modest amounts of nitrate also are leached from soil under natural conditions or from undisturbed soil that are well-covered with native vegetation.

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Agrovoc descriptors: foods; demand; food consumption; commodity markets; price elasticities; expenditure; consumer expenditure; family budget; household consumption; living standards; feeding habits; cultural behaviour; consumer behaviour; value systems

Agris category codes: E73, E50, E70

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Food demand in Slovenia

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ABSTRACT

The objective of this research is to analyse food consumption patterns in Slovenia. Cross-sectional household data from Household budget survey in year 2001 were used. We estimate expenditure and price elasticities for food demand for households segmented by quartile income levels and for Slovenia total. Food items are divided into the following commodity groups: bread and cereals, meat and fish, dairy products, oils and other fats, fruit, vegetables, confectionary. For a complete demand system analysis, we apply linearly approximated Almost Ideal Demand System (LA/AIDS). Empirical results show positive expenditure elasticities being close to one for all food groups. In general, demands for dairy products and vegetables could be regarded as the most sensitive to food expenditures. Further on, all Marshallian (uncompensated) and Hicksian (compensated) own price elasticities are negative and less than one. Own price elasticities for meat and fish are estimated as the lowest and for vegetables as the highest. With cross price elasticities close to zero the studied commodity groups seem to be unrelated. The negative sign of uncompensated cross price elasticities indicates complementary type of food groups, while substitution relationship of aggregate foods is indicated by mostly positive Hicksian cross price elasticities. According to these expenditure and price elasticities inhabitants of Slovenia seem to be losing consumption characteristics typical for countries in transition. However some unique food habits persist.

Key words: food demand / commodity groups / demand system / LA/AIDS / expenditure and price demand elasticities / Household budget survey / income groups

IZVLEČEK

POVPRAŠEVANJE PO HRANI V SLOVENIJI

Namen študije je analiza prehranskih navad prebivalcev Slovenije. Podatke iz Ankete o porabi v gospodinjstvih v letu 2001 smo uporabili za ocenjevanje izdatkovnih in cenovnih elastičnosti povpraševanja po hrani za gospodinjstva v Sloveniji. Gospodinjstva so razdeljena v kvartilne dohodkovne razrede in kot Slovenija skupaj. Prehranske izdelke smo razdelili v naslednje skupine živil: kruh in žita, meso in ribe, mlečni izdelki, olja in druge maščobe, sadje, zelenjava, sladki izdelki. Povpraševanje po hrani je ocenjeno kot sistem enačb povpraševanja z linearno aproksimacijo metode skoraj idealni sistem povpraševanja (LA/AIDS). Ocenjene

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izdatkovne elastičnosti so pozitivne, z vrednostmi blizu 1. V splošnem lahko trdimo, da sta povpraševanje po mlečnih izdelkih ter povpraševanje po zelenjavi najbolj občutljivi na spremembe v izdatkih namenjenih hrani. Nadalje, vse Marshallove (nekompenzirane) in Hicksove (kompenzirane) lastne cenovne elastičnosti so negativne in manjše od 1. Lastne cenovne elastičnosti povpraševanja po mesu in ribah so bile ocenjene kot najnižje, povpraševanja po zelenjavi pa kot najvišje. Omenjene skupine živil so med seboj cenovno nepovezane, saj so križne cenovne elastičnosti blizu vrednosti nič. Večina nekompenziranih križnih cenovnih elastičnosti je negativna, kar nakazuje na komplementarnost proučevanih skupin živil oziroma na substitute, ko govorimo o večinoma pozitivnih Hicksovih križnih cenovnih elastičnostih. Ocenjene izdatkovne in cenovne elastičnosti kažejo, da prebivalci Slovenije izgubljajo prehranske navade značilne za države v tranziciji. Kljub temu nekatere prehranske posebnosti ostajajo.

Ključne besede: povpraševanje po hrani / skupine živil / sistem enačb povpraševanja / LA/AIDS / izdatkovne in cenovne elastičnosti povpraševanja / Anketa o porabi v gospodinjstvih / dohodkovni razredi

INTRODUCTION

When estimating demand for goods and services studies reveal two approaches. Single equation approach specifies demand model directly and does not rely on economic theory of consumer behaviour which recognises the importance of income and prices. For example, Engel curves the most frequently used method ignores price influence. Given the doubts for results of such approach, empirical work has been directed towards the estimation of complete demand systems derived from consumer theory. Modern studies calculate responsiveness of individual consumer behaviour to prices faced by households and to the income they earn (Blanciforti *et al.*, 1986).

Until 90s all food demand studies in Slovenia applied single equations approach, more precisely Engel curves method (Frankovič, 1958 (cited in Kranjec, 1981); Verk, 1969; Kranjec, 1981; Kebrič, 1981; Šumi, 1986; Regoršek, 2002). Erjavec and Turk (1998) carried out the first study which estimated the effect of income and prices on food demand in Slovenia simultaneously. They estimated Slovene food demand functions as a system and Slovene food demand elasticities in years 1988 and 1993.

Our study updates earlier food demand studies for Slovenia in two ways. First and foremost, we present recent estimates of Slovene income and price elasticities for seven food commodity aggregates. Secondly, this study estimates food demand as a system of equations. We apply LA/AIDS method. While time-series data on demand for food categories and their prices are not available in Slovenia we use individual household data from Household budget survey 2001.

MODEL

Deaton and Muellbauer introduced Almost Ideal Demand System (AIDS) model in 1980. The model is derived from a flexible expenditure function that is extremely useful for estimating a demand system with many desirable properties. Aggregation restriction (Muellbauer, 1975) and simple parametric restrictions are automatically satisfied, homogeneity and symmetry can be imposed. Its functional form is consistent with known household budget data. Owing to its simplicity, the Linear

Approximate Almost Ideal Demand System (LA/AIDS) model is very popular for empirical studies (Deaton and Muellbauer, 1980 and 1980a; Phlips, 1990; Alston *et al.*, 1994; Peterson and Cotterill, 1998). Its estimation is much simpler because linear estimation procedures can be used. LA/AIDS demand functions have the form:

$$w_i = \alpha_i + \sum_j \gamma_{ij} \ln p_j + \beta_i \ln(x/P^*) \quad [1]$$

where $\alpha_i, \beta_i, \gamma_{ij}$ are parameters, w_i is budget share of good i ($i=1,2,\dots,n$), p_j is price of good j ($j=1,2,\dots,n$) and x denotes total expenditure. P^* is Stone's price index defined as:

$$\ln P^* = \sum_j w_j \ln p_j \quad [2]$$

On the parameters of LA/AIDS model the following restrictions can be imposed (Jehle and Reny, 2001):

1. Adding-up implies the following parameters' restrictions

$$\sum_{i=1}^n \alpha_i = 1 \quad \sum_{i=1}^n \beta_i = 0 \quad \sum_{i=1}^n \gamma_{ij} = 0. \text{ Hence it follows that } \sum w_i = 1 \text{ as it is clear from [1].}$$

2. Homogeneity requires that $\sum_{i=1}^n \gamma_{ij} = 0$.
3. Symmetry is satisfied if $\gamma_{ij} = \gamma_{ji}$ for any two goods i and j .
4. Negativity is not automatically introduced, but by estimating all the compensated own-price elasticities one can test for their negativity.

According to Green and Alston (1990), elasticities in LA/AIDS model can be expressed as $\eta_i = 1 + \beta_i/w_i$ for income elasticity and $e_i = -\delta_{ij} + \gamma_{ij}/w_i - \beta_i(w_j/w_i)$ for uncompensated (Marshallian) price elasticity where $\delta_{ij} = 1$ if $i = j$ and $\delta_{ij} = 0$ if $i \neq j$. A total price change effect is a sum of price effect and income effect which together affect on quantity demanded. When we are interested only in income effect of a price change assuming price effect is constant, compensated (Hicksian) price elasticities should be calculated. In LA/AIDS model that are as $e_{ij}^* = e_{ij} + w_j \eta_i$ (Hahn, 1994).

In LA/AIDS model parameter β_i determines the effect of a change in expenditure on the budget share of good i and whether this good is a luxury, necessity or inferior good. For a luxury $\beta_i > 0$, expenditure elasticity is larger than unity ($\eta_i > 1$) indicating w_i increases when total expenditures are rising (x). For a necessary good $\beta_i < 0$, expenditure elasticity lies between zero and unity ($0 < \eta_i < 1$) meaning w_i decreases when x increases. And for an inferior good $\beta_i < -1$, with expenditure elasticity smaller than zero ($\eta_i < 0$). In addition, it is possible to examine all complementary and

substitutive relations between pairs of goods by estimating compensated and uncompensated cross price elasticities (Varian, 1992).

DATA

Slovenian Household budget survey conducted annually by Statistical Office of the Republic of Slovenia was main data source for this study. First similar survey was conducted in 1983 and since 1997 this survey is harmonized with Eurostat's standards. It is a sample survey. To obtain more accurate estimates household data from three consecutive yearly surveys are combined in one sample. Implicitly, all value data have to be deflacionated, namely to the middle year which is quoted as a reference year when interpreting results. We used Household budget survey data with reference year in year 2001. It was carried out in years 2000, 2001 and 2002 and covered 4986 households, of that 3816 households responded (Statistical yearbook of the Republic of Slovenia 2004).

Among others, survey gathers data on total food expenditure, quantity and value of specific food items consumed by a household. Food items are aggregated into the following 7 composite food categories: bread and cereals, meat and fish, dairy products, oils and other fats, fruit, vegetables and confectionary. Aggregation across food commodities revealed households which reported no consumption of particular food group(s). For such households average quantity consumed and average expenditure of non consumed aggregated food categories are used in LA/AIDS estimation. Since the household survey does not report price data, unit values as price indicators for aggregated food groups are calculated across households. They are derived as weighted average of each food item consumed and its quantity in the food group the item belongs to. Weights represent relative consumption share of each food item in total consumption of its aggregated food group.

Households are classified into quartile income groups (borders of each income group are the same as quartile borders) according to the value of average annual disposable income per household member. All entry data for further food demand analysis are calculated per household member.

RESULTS

LA/AIDS model [1] is estimated for five household samples: four quartile income groups and all households in Slovenia (hereafter, Slovenia total). Models are estimated as a system of linear equations, using the systems linear regression (SYSLIN) procedure in SAS computer programs (Zellner, 1962). The parametric constraints of homogeneity and symmetry conditions across the equations are imposed. To avoid singularity derived from adding-up constraint in the variance-covariance matrix one equation ("confectionary" in our case) is deleted from direct estimation in each demand system. The parameters' estimates of confectionary equation are derived using homogeneity, symmetry and adding-up conditions. The share of estimated demand coefficients, which p -value is less than 0.1 varies, according to the model, between 70% and 81%.

However, of interest to researchers and policy makers is knowledge concerning elasticities of demand for food. According to the value of expenditure elasticities, selected food groups can be classified as inferior goods ($\eta_i < 0$), necessities ($0 < \eta_i < 1$) and luxuries ($\eta_i > 1$). It should be noted that food group indicated as luxury/necessity/inferior good is regarded as luxury/necessity/inferior commodity according to total food expenditure and not according to total household expenditures. It can be seen from Table 1 that all expenditure elasticities are of expected sign and close to 1. Values in parentheses are p -values testing $H_0 : \eta_i = 1$. Demand for dairy products and demand for vegetables could be regarded as the most expenditure elastic, statistically significant at 5 percent level. All other food groups are fairly sensitive to food expenditure changes. Estimates of expenditure elasticities segmented by income groups show that demand for bread and cereals which expenditures represent about 20% of total food expenditures could also be regarded as very elastic for low income households yet it is not statistically significant. Households in the second and the fourth income group treat this food group as necessity but again this cannot be statistically proven. Fruit demand is similarly unusual with relatively high expenditure elasticities for high income households but are not statistically significant.

Table 1. Expenditure elasticities of aggregated food groups, η_i , and expenditure shares of aggregated food groups in total food expenditures, w_i , (in %), four quartile income groups of households in Slovenia and Slovenia total, 2001.

Preglednica 1. Izdatkovne elastičnosti agregatnih skupin živil, η_i , in deleži izdatkov agregatnih skupin živil v skupnih izdatkih za hrano, w_i , (v %), štiri kvartilni dohodkovni razredi gospodinjstev v Sloveniji in Slovenija skupaj, 2001.

i	1 st (low) income households		2 nd income households		3 rd income households		4 th (high) income households		Slovenia total	
	w_i	η_i	w_i	η_i	w_i	η_i	w_i	η_i	w_i	η_i
1	20.4	1.026 (0.2098)	18.2	0.994 (0.4198)	17.5	0.929 (0.0100)	16.4	0.966 (0.4426)	18.1	0.961 (0.0041)
2	26.4	0.924 (0.0099)	30.5	0.938 (0.0153)	29.9	0.930 (0.0109)	29.2	0.968 (0.1291)	29.0	0.952 (0.0005)
3	21.5	1.093 (0.0046)	21.8	1.141 (<0.0001)	22.0	1.170 (<0.0001)	23.6	1.063 (0.0173)	22.2	1.121 (<0.0001)
4	5.4	0.729 (<0.0001)	5.2	0.670 (<0.0001)	5.1	0.677 (<0.0001)	5.0	0.726 (<0.0001)	5.2	0.708 (<0.0001)
5	8.4	0.897 (0.0240)	8.1	0.841 (0.0005)	8.5	1.023 (0.3204)	9.2	1.004 (0.4653)	8.6	0.967 (0.0830)
6	9.9	1.234 (<0.0001)	9.3	1.226 (<0.0001)	9.8	1.141 (0.0038)	9.7	1.150 (0.0005)	9.7	1.180 (<0.0001)
7	8.0	0.939	6.8	0.976	7.2	0.953	6.8	0.912	7.2	0.927

Legend

i: food group

1: Bread and cereals

2: Meat and fish

3: Dairy products

4: Oils and other fats

5: Fruit

6: Vegetables

7: Confectionary

According to the Table 2 all uncompensated own price elasticities (e_{ii}) are negative and less than 1. Demand for bread and cereals and demand for meat and fish seem to be the least sensitive to its own price changes. On the other hand, households tend to respond very rapidly to price changes in dairy products and vegetables when demanding them. Uncompensated own price elasticities do not vary systematically across income groups.

Hicksian own price elasticity estimates (e_{ii}^*) in Table 2 have similar trend and as derived from theory also have smaller values as Marshallian ones. The estimates of compensated own price elasticities for bread and cereals, dairy products and especially for meat and fish are noticeably smaller than uncompensated. This indicates that income effect of their price change on their own quantity demanded is highly important when purchasing these food groups (Figures 1-5). Demand for meat and fish is almost inelastic to its own price changes. It is unusual that the lowest income households increase their purchases of meat and fish when these prices rise (positive sign of Hicksian own price elasticity). And again, own price elasticity for vegetables is the highest regardless of constant price effect of its price change.

Table 2. Uncompensated, e_{ii} , and compensated, e_{ii}^* , own price elasticities for aggregated food groups, four quartile income groups of households in Slovenia and Slovenia total, 2001.

Preglednica 2. Nekompenzirane, e_{ii} , in kompenzirane, e_{ii}^* , lastne cenovne elastičnosti agregatnih skupin živil, štirje kvartilni dohodkovni razredi gospodinjstev v Sloveniji in Slovenija skupaj, 2001.

i	1 st (low) income households		2 nd income households		3 rd income households		4 th (high) income households		Slovenia total	
	e_{ii}	e_{ii}^*	e_{ii}	e_{ii}^*	e_{ii}	e_{ii}^*	e_{ii}	e_{ii}^*	e_{ii}	e_{ii}^*
1	-0.539	-0.330	-0.479	-0.291	-0.467	-0.287	-0.438	-0.269	-0.474	-0.299
2	-0.206	0.038	-0.374	-0.092	-0.340	-0.064	-0.397	-0.128	-0.332	-0.056
3	-0.842	-0.607	-0.821	-0.583	-0.817	-0.576	-0.766	-0.508	-0.814	-0.565
4	-0.596	-0.556	-0.630	-0.592	-0.512	-0.475	-0.539	-0.502	-0.570	-0.534
5	-0.579	-0.503	-0.508	-0.436	-0.578	-0.502	-0.412	-0.329	-0.520	-0.437
6	-0.901	-0.779	-0.924	-0.809	-0.785	-0.664	-0.794	-0.674	-0.852	-0.737
7	-0.596	-0.521	-0.645	-0.581	-0.535	-0.468	-0.551	-0.487	-0.576	-0.509

Legend

i: food group	4: Oils and other fats
1: Bread and cereals	5: Fruit
2: Meat and fish	6: Vegetables
3: Dairy products	7: Confectionary

Our study contains uncompensated and compensated cross and own price elasticities although only the latter are presented in this paper (Table 2). With cross price elasticities close to zero most of the food groups seem to be unrelated. Uncompensated cross price elasticities are mostly negative indicating complementary type of food groups. Hicksian cross price elasticities have mostly positive sign suggesting substitution relationship of aggregated foods.

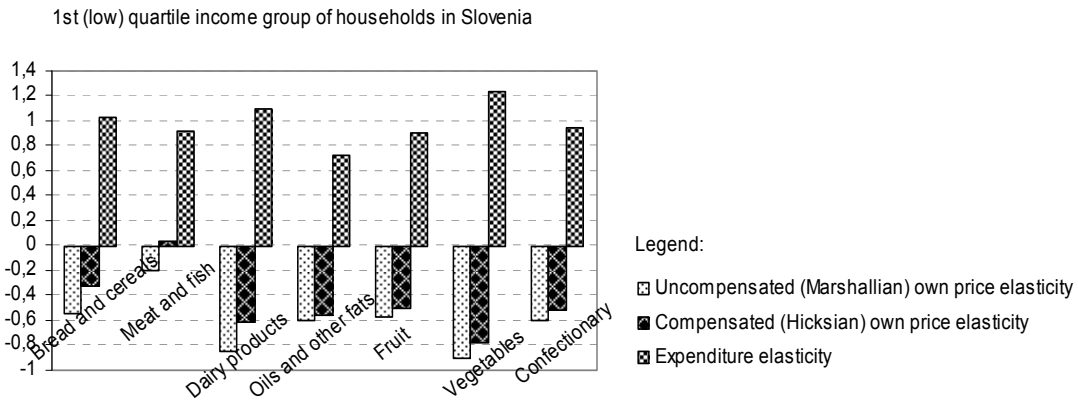


Figure 1. Uncompensated, e_{ii} , and compensated, e_{ii}^* , own price elasticities and expenditure elasticities, η_i , for aggregated food groups, 1st (low) quartile income group of households in Slovenia, 2001.

Slika 1. Nekompenzirane, e_{ii} , in kompenzirane, e_{ii}^* , lastne cenovne elastičnosti in izdatkovne elastičnosti, η_i , agregatnih skupin živil, prvi (nižji) kvartilni dohodkovni razred gospodinjstev v Sloveniji, 2001.

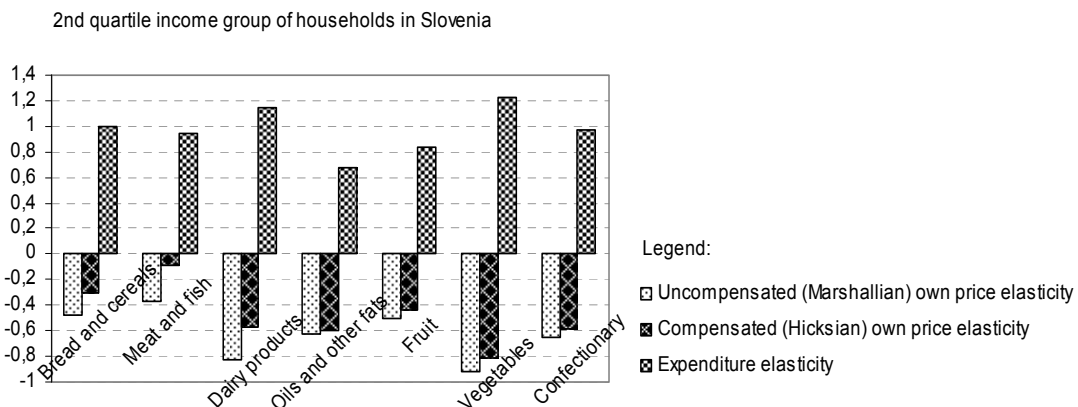


Figure 2. Uncompensated, e_{ii} , and compensated, e_{ii}^* , own price elasticities and expenditure elasticities, η_i , for aggregated food groups, 2nd quartile income group of households in Slovenia, 2001.

Slika 2. Nekompenzirane, e_{ii} , in kompenzirane, e_{ii}^* , lastne cenovne elastičnosti in izdatkovne elastičnosti, η_i , agregatnih skupin živil, drugi kvartilni dohodkovni razred gospodinjstev v Sloveniji, 2001.

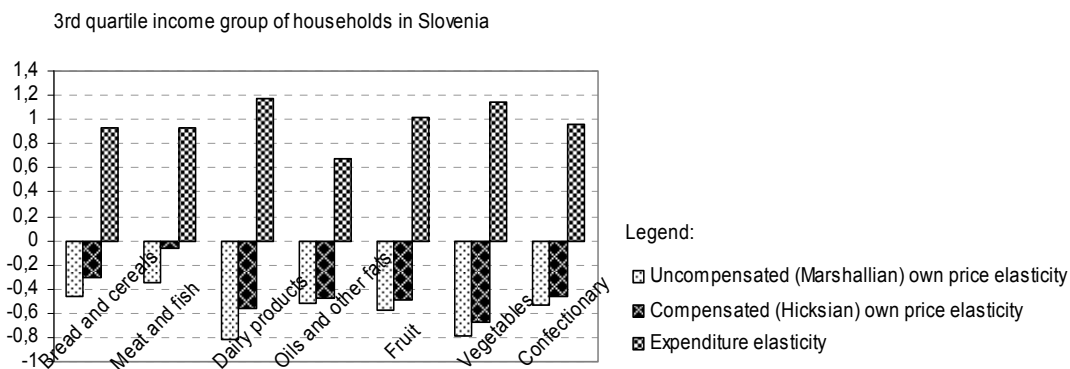


Figure 3. Uncompensated, e_{ii} , and compensated, e_{ii}^* , own price elasticities and expenditure elasticities, η_i , for aggregated food groups, 3rd quartile income group of households in Slovenia, 2001.

Slika 3. Nekompenzirane, e_{ii} , in kompenzirane, e_{ii}^* , lastne cenovne elastičnosti in izdatkovne elastičnosti, η_i , agregatnih skupin živil, tretji kvartilni dohodkovni razred gospodinjstev v Sloveniji, 2001.

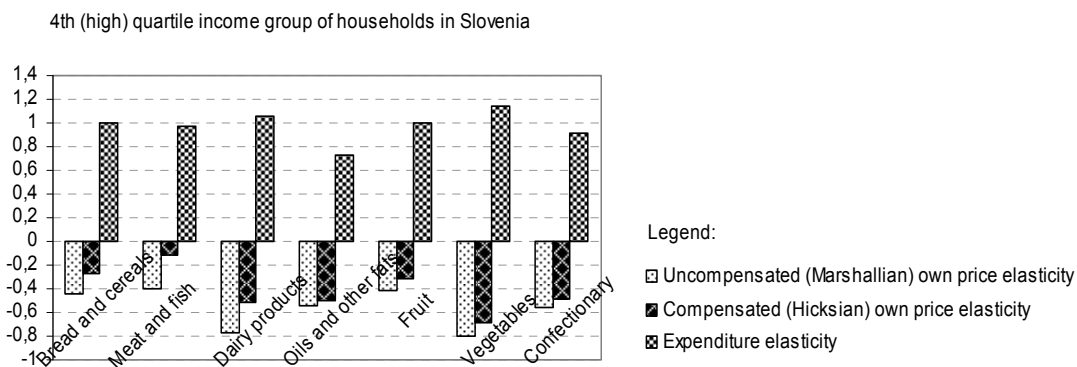


Figure 4. Uncompensated, e_{ii} , and compensated, e_{ii}^* , own price elasticities and expenditure elasticities, η_i , for aggregated food groups, 4th (high) quartile income group of households in Slovenia, 2001.

Slika 4. Nekompenzirane, e_{ii} , in kompenzirane, e_{ii}^* , lastne cenovne elastičnosti in izdatkovne elastičnosti, η_i , agregatnih skupin živil, četrti (višji) kvartilni dohodkovni razred gospodinjstev v Sloveniji, 2001.

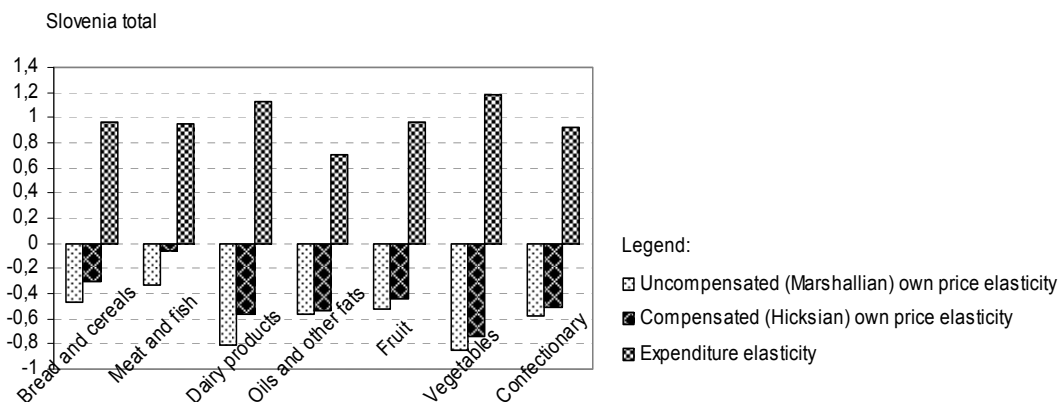


Figure 5. Uncompensated, e_{ii} , and compensated, e_{ii}^* , own price elasticities and expenditure elasticities, η_i , for aggregated food groups, Slovenia total, 2001.

Slika 5. Nekompenzirane, e_{ii} , in kompenzirane, e_{ii}^* , lastne cenovne elastičnosti in izdatkovne elastičnosti, η_i , agregatnih skupin živil, Slovenija skupaj, 2001.

CONCLUSION

In this study we modelled food demand in Slovenia as a system of equations using LA/AIDS method (Deaton and Muellbauer, 1980). To investigate consumer purchasing habits in Slovenia we estimated demand elasticities for four quartile income groups of households and for all households (Slovenia total) in year 2001.

Our results confirm findings (Huang and Lin, 2000; Conforti et al., 2000; Erjavec and Turk; 1998) that items in bread and cereals group are treated as a normal good, which is relatively insensitive to its own price changes. A characteristic typical for lower income countries (Katchova and Chern, 2004) still appeared in post-transition Slovenia that is, the lowest income households in Slovenia treat these items as luxury goods. Remaining groups of households regard this food group as a normal good. However, both conclusions cannot be statistically proven. These findings could be explained with rising trend in consumption of bread and cereals (Duffy, 2001) or with a shift in consumption of different items within this food group (Abdulah et. al., 1999).

Decreasing food elasticity of meat and fish demand in Slovenia since 1988 is an obvious example of consumption patterns' changes from ones distinctive of transition countries (Erjavec and Turk, 1998; Stavrev and Kambourov, 1999) towards ones typical of developed countries (Duffy, 2001; Ledezma et al., 2002; Huang and Lin, 2000). This weak responsiveness to food expenditures and its own price changes is mainly due to constant (Duffy, 2001; Ledezma et al., 2002; Huang and Lin, 2000) or, in our case, decreasing (Volk, 2004) share of meat consumption.

Since 1988 consumers in Slovenia have been increasing purchases of dairy products when their income has been increasing. This country specific consumption pattern,

which is similar to that in France, might be explained with relatively high demand share of dairy products (Ledezma et al., 2002). On the other hand, own price elasticities have been rising ever since. Consumption shifts within this food group might have caused this trend (Huang and Lin, 2000) but only further analysis could confirm that.

Characteristics in fruit demand and vegetables demand have drastically changed in 2001 compared to Slovenia's transition period (Erjavec and Turk, 1998) as well as compared to other countries (Ledezma et al., 2002; Huang and Lin, 2000; Katchova and Chern, 2004; Abdulah et al., 1999). In 2001 fruit was treated as normal good and vegetable as luxury good. Ledezma et al. (2002) explained this peculiarity from supply side point of view: most of the fruit is imported, whereas vegetables are produced domestically. On the contrary, in 2001 demand for vegetables was more sensitive to its own price changes than demand for fruit which is again just the opposite to findings of countries concerned (Ledezma et al., 2002; Huang and Lin, 2000; Katchova and Chern, 2004; Abdulah et al., 1999). From Slovene transition period own price elasticities for fruit have dropped even further in 2001, while vegetables' own price elasticities have grown noticeably. This outcome might be explained with the assumption that share of home produced vegetables in Slovenia is higher than home production of fruit. Nevertheless, consumption of fruit in Slovenia has been rising quicker than consumption of vegetables (Volk, 2004) implying that consumption habits in demands of fruit and vegetables in Slovenia are changing.

Knowledge of demand elasticities and their trends gives us an overview of consumption habits of a certain population. This is an important information for policy makers (food policy), researchers (sector analysis) and companies (pricing policy, marketing actions) so reliable food demand parameters are fundamental for further analysis. Applying LA/AIDS method enabled us to gain more reliable food demand parameters than those obtained with Engel curves method as it was a long Slovenian experience in the past. Nevertheless, there are still some limitations of this study. As all (ex) countries in transition Slovenia lacks of appropriate data. In our case price data for seven food groups had to be calculated across households. Broad span of these values indicated that quality of food is important but was ignored in the study. This implies that price elasticities would probably be different when quality impact would have been excluded (Stavrev and Kambourov, 1999). Another disadvantage that rises from data shortage is a choice of alternative method. LA/AIDS model has been constantly improving but recent models claim for time series data (e.g. dynamic AIDS) or more disaggregated data (e.g. quadratic AIDS), which are not available in Slovenia yet. Further food demand analysis applying disaggregated data of food group would be interesting as it could reveal if changes within each group really imply higher demand elasticities as it is usually the case in developed countries. Factors such as consumer's age, field of occupation, number of children, etc. proved to be more important than income (expenditure) level and prices when demanding different food items (Conforti et al., 2000). Since Slovene household budget survey does contain non economic factors further analysis should study their impact on food demand in Slovenia.

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Agrovoc descriptors: zea mays; land varieties; plant population; gene banks; genetic markers; plant anatomy; crossbreds; random mating; kernels; colour

Agris category codes: F30, F40, F50

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COBISS koda: 1.0

Ocena dveh različnih skupin slovenskih populacij koruze s pomočjo morfoloških lastnosti in *Hbr* (MITE) markerjev

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IZVLEČEK

Analizirali smo dve skupini slovenskih populacij koruze iz genske banke Oddelka za agronomijo Biotehniške fakultete v Ljubljani. V prvo skupino smo uvrstili enajst populacij koruze iz Bohinja (B1-B9 in B11-B12), v drugo štirinajst štajerskih populacij, z okolice Vojnika in Laškega (Š1-Š14). Petintrideset morfoloških lastnosti rastlin, storžev in zrnja smo izvedli v skladu z mednarodnimi deskriptorji IPGRI v času rastle dobe na polju in v laboratoriju. Genetski opis je temeljil na 161 *Heartbreaker* (*Hbr*) markerjih, pri čemer je bila analiza izvedena na skupnih vzorcih DNA, po 25 rastlin iz vsake populacije. Populacije iz Bohinja so se na splošno dobro razlikovale od štajerskih populacij, tako po morfoloških lastnostih kot na osnovi genetskih markerjev. Izjemi sta le populaciji B1 in B8, ki sta po morfoloških znakih še najbolj podobni štajerskim populacijam. Glede na posamezne morfološke lastnosti B1 odstopa od drugih bohinjskih populacij le po barvi zrnja, ki je ob brezbarvnem perikarpu pogojena z barvo alevrona. Je edina bohinjka z rumenim zrnjem, vse ostale bohinjske populacije imajo bakreno barvo zrnja, medtem ko imajo štajerske populacije rumeno ali belo zrnje. Glede na vse analize, še posebej glede na prisotnost/odsotnost *Hbr* elementov ter njihove frekvence v populacijah, lahko kljub nekaterim podobnostim med posameznimi populacijami trdimo, da gre za različne populacije, čeprav nekatere izhajajo iz lokacij, ki so si geografsko zelo blizu. Torej so posamezni pridelovalci s svojim vzdrževanjem in ponovno setvijo lastnega semena uspeli obdržati karakteristične lastnosti svoje populacije, s tem da so preprečili nekontrolirano križanje z drugimi populacijami.

Ključne besede: *Zea mays* / populacije / IPGRI deskriptorji / morfologija / *Hbr* markerji / genetsko razmerje

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ABSTRACT

EVALUATION OF TWO DIFFERENT GROUPS OF SLOVENIAN MAIZE POPULATIONS USING MORPHOLOGICAL TRAITS AND *Hbr* (MITE) MARKERS

Two different groups of Slovenian maize populations from the germplasm bank at the Agronomy Department of Biotechnical Faculty in Ljubljana were analyzed. The first group included eleven landraces from Bohinj (B1-B9 and B11-B12) and the second one included 14 landraces from Štajerska region, from the surroundings of Vojnik and Laško (Š1-Š14). Thirty-five morphological characteristics of plants, ears and kernels were described using IPGRI (International Plant Genetic Resource Institute) descriptors during the vegetation period on a field and in a laboratory. Genetic characterization was performed on DNA bulks from 25 plants per population by genotyping 161 *Heartbreaker* (*Hbr*) markers. Generally, according to the genetic and morphological data, populations from Bohinj are clearly differentiated from populations from Štajerska region. The only exceptions are populations B1 and B8, which are similar to Štajerska populations, according to the morphological traits. B1 differs from other Bohinj populations by color of kernels, which is yellow due to the colorless pericarp and yellow aleuron. Kernels of all other Bohinj population had brown color, while Štajerska populations had yellow or white kernels. Analysis, especially presence/absence of *Hbr* elements and their population frequencies, suggests that all populations are separated, although some of them are geographically closely located. It seems that farmers have been able to maintain their landraces by repeat sowing of their own seeds and by preventing uncontrolled crossing with other populations.

Key words: *Zea mays* / populations / IPGRI descriptors / morphology / *Hbr* markers / genetic relationship

UVOD

Genetska raznolikost žlahtniteljskega materiala je eden od najpomembnejših dejavnikov, ki vplivajo na uspeh žlahtnjenja rastlin. Pri koruzi je heterotičen učinek, ki se izkorišča v hibridih F1 generacije, odvisen od genetske raznolikosti starševskih linij, kar sta ugotovila že East (1908) in Shull (1908). Ker se v intenzivnih žlahtniteljskih programih kot starševske linije večinoma uporablja elitne linije, prihaja do oženja genetske raznolikosti, kar lahko privede do izgube določenih pomembnih genotipov. Za ugotavljanje raznolikosti poleg morfoloških lastnosti lahko uporabljamo še različne DNK markerje, npr. mikrosatelite, AFLP in pri koruzi tudi *Hbr* markerje. Slednje so razvili Casa in sod. (2000) in so specifični za koruzo, visoko polimorfni in enakomerno razporejeni po celem genomu (Casa in sod., 2002). Temeljijo na prisotnosti/odsotnosti miniaturnih transponirajočih elementov z invertiranimi ponavljajočimi sekvencami (MITE) iz družine *Heartbreaker* na določeni lokaciji v genomu. Tipiziramo jih s pomočjo tehnike *Hbr* prikaz, ki je podobna AFLP tehniki, le da je en začetni nukleotid zasidran v restriksijsko mesto encima *MseI*, drug pa v transponirajoči element (Casa in sod., 2000).

V genski banki koruze Oddelka za agronomijo na Biotehniški fakulteti v Ljubljani se ohranja več kot 516 genotipov koruze, od katerih je polovica večinoma slovenskih populacij, polovico pa zajemajo iz njih vzgojene bolj ali manj homozigotne linije (Rozman, 1998). V naši prejšnji raziskavi smo petnajst pomembnejših slovenskih populacij koruze ocenili s *Hbr* markerji (Kavar in sod., 2007). Vseh deset vzorcev iz iste populacije je imelo zelo podobne DNK profile, medtem ko so bile razlike med populacijami večje; z analizo AMOVA je bil delež variance, ki izhaja iz razlik med

populacijami ocenjen na 76,7%. Po pričakovanju, je od vseh populacij najbolj odstopala edina zobanka (beli zob), izmed trdink pa populacija štajerski dvanajsterec.

Ker so se *Hbr* markerji v prejšnji raziskavi (Kavar in sod., 2007) izkazali za primerne za karakterizacijo populacij koruze, smo jih tudi v tem delu, skupaj z morfološkim opisom (deskriptorji IPGRI), uporabili za oceno enajstih populacij z Bohinja in štirinajstih populacij s Štajerske. Namen je bil ugotoviti sorodnost tako med različnima skupinama populacij kot tudi med populacijami znotraj ene skupine, kar bi predstavljalo pomembno informacijo za načrtovanje učinkovitejše uporabe slovenske dednine koruze v žlahtnjenju.

MATERIAL IN METODE

Populacije koruze

V proučevanje smo vključili dve skupini populacij koruze iz genske banke koruze Oddelka za agronomijo Biotehniške fakultete v Ljubljani. Prvo skupino predstavlja enajst populacij koruze, ki izhajajo od različnih pridelovalcev koruze iz Bohinja (B1-B9 in B11-B12). Drugo skupino pa predstavlja štirinajst štajerskih populacij iz okolice Vojnika in Laškega (Š1-Š14) (Pregl. 1). Populacije smo pridobili s terenskim nabiranjem vzorcev v letih 1986 do 1988 (bohinjske populacije) oz. v letih 1989 do 1990 (štajerske populacije).

Preglednica 1: Izvor proučevanih populacij.

Table 1: The origin of investigated populations.

Populacija	Kraj	Nadm. višina (m)
Population	Location	A.s.l. (m)
B1, B3, B7	Srednja vas	570
B8	Češnjica	615
B4, B5, B11	Studor	590
B2	Jereka	670
B6, B12	Stara Fužina	550
B9	Podjelje	800
Š1, Š2, Š4	Višnja vas	300
Š6	Polže	290
Š8	Hrenova – Strmec	290
Š10	Razdelj	290
Š13	Mačkovec – Laško	625
Š11, Š12	Olešče – Laško	480
Š3	Mala Breza	550
Š5, Š7, Š9	Breze – Šentrupert	570
Š14	Šentjur pri Celju	260

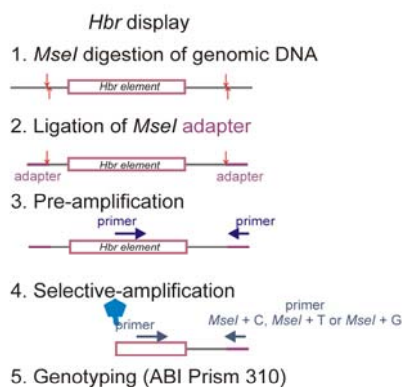
Morfološko-agronomske lastnosti

Poljski poskus za opis morfoloških lastnosti proučevanih populacij je bil izveden na poskusnem polju Biotehniške fakultete na Centru za razvoj kmetijstva in podeželja v Jablah pri Mengšu. V času vegetacije so bile na polju v skladu z deskriptorji IPGRI-ja na 20 rastlinah opravljene meritve in opisi morfoloških lastnosti rastlin. Meritve in opisi storžev in zrnja teh rastlin so bile opravljene po spravilu, v laboratoriju Oddelka za agronomijo Biotehniške fakultete v Ljubljani.

Razlike med posameznimi rastlinami smo ocenili s koeficienti podobnosti, po metodi, ki jo je opisal Gower (1971), ker le-ta dovoljuje uporabo podatkov mešanega tipa. Podatke o 35 morfoloških lastnostih smo najprej porazdelili glede na tip podatkov v tri skupine: 21 lastnosti med podatke intervalnega tipa, sedem med podatke ordinalnega in sedem med nominalne (simetrične) podatke. Za izračun koeficienta podobnosti, PCA analizo in prikaz tridimenzionalnega grafa prvih treh glavnih komponent (Sl. 2) smo uporabili SAS V8 software (SAS Institute Inc.). Drugo PCA analizo (Sl. 3) smo izvedli na podlagi povprečnih vrednosti v populacijah za 21 kvantitativnih lastnosti.

Genetski opis

Po en skupen DNK vzorec za vsako populacijo smo izolirali iz listov petindvajsetih rastlin koruze z Gen Elute Plant Genomic DNA Miniprep kitom (Sigma). *Hbr* prikaz smo izvedli po že opisani metodi (Casa in sod., 2000) (Sl. 1). Okrog 500 ng genomske DNA smo razrezali z restriksijskim encimom *MseI* - dve uri pri 65 °C v 20 µl reakciji, ki je vsebovala 2 x Tango pufer in 1 U *MseI* encima (Fermentas). Sledila je ligacija adapterjev (5'-GACGATGAGTCCTGAG in 5'-TACTCAGGACTCAT), z dodajanjem 5 µl mešanice, ki je vsebovala 1x ligacijski pufer, 25 pmol adapterjev in 0,5 U T4 DNA ligaze (Fermentas) ter inkubacija - tri ure pri 37 °C. Za pre-amplifikacijo smo uporabili začetna oligonukleotida: *MseI*+O (5'-GACGATGAG-TCCTGAGTAA) in *Hbr*-Int5-E (5'-GATTCTCCCCACAGCCAGATTC). Selektivno amplifikacijo smo izvedli s tremi kombinacijami začetnih oligonukleotidov: fluorescentno označen *Hbr*-Int5-F (5'-FAM-GAGCCAGATTTTCAGAAAAGCTG) z enim izmed treh selektivnih oligonukleotidov: *MseI*+C, *MseI*+G in *MseI*+T. Denaturirane PCR produkte smo ločili s kapilarno elektroforezo na avtomatskem sekvenatorju ABI PRISM 310 (PE Applied Biosystems), pri čemer je bil vsakemu vzorcu dodan velikostni standard GeneScan Rox 500 (Applied Biosystems).



Slika 1: Shema metode »*Hbr* prikaz« po Casa in sod., (2000). *Hbr* metoda je podobna metodi AFLP, le da je en začetni oligonukleotid zasidran v restriksijsko mesto *MseI*, drug pa v transponirajoči element. 1. DNK razrežemo z restriksijskim encimom *MseI*. 2. Z ligacijo dodamo adapterje na restriksijsko mesto za *MseI*. 3. Pre-amplifikacija. 4. Selektivna amplifikacija. 5. Tipizacija *Hbr* elementov s pomočjo avtomatskega sekvenatorja ABI Prism 310 (PE Applied Biosystems).

Figure 1: Sheme of the *Hbr* display technique (developed by Casa et al., 2000). *Hbr* display is the modification of the AFLP technique where the PCR products are derived from primers anchored in a restriction site (*i.e.* *MseI*) and a transposable element of maize MITE family *Heartbreaker* rather than in two restriction sites.

V tipizacijo smo vključili samo PCR produkte (*Hbr* elemente), ki niso bili problematični za tipizacijo. Zbrane podatke o višini vrha za posamezne PCR produkte smo standardizirali in jih uporabili za oceno pogostosti določenega *Hbr* elementa v posamezni populaciji. Na podlagi teh ocen smo s programi Seqboot, Gendist, Neighbor in Consense iz programskega paketa Phylip (Phylogeny Inference Package) verzija 3.6. (Felsenstein, 2005) izrisali drevo in z analizo "bootstrap" statistično ovrednotili topologijo drevesa. Drevo (Sl. 4) smo grafično predstavili s pomočjo programa TreeView verzija 1.6.6 (Page, 2001). S SAS V8 software (SAS Institute Inc.) smo na podlagi genetskih podatkov izračunali koeficiente podobnosti med populacijami po metodi Simratio, izvedli PCA analizo in grafično predstavili rezultate s tridimenzionalnim grafom prvih treh glavnih komponent (Sl. 5).

REZULTATI IN RAZPRAVA

Morfološko-agronomske lastnosti

V proučevanje je bilo vključenih 35 morfoloških lastnosti rastlin, storžev in zrnja, za katere smo v skladu z deskriptorji IPGRI-ja opravili ustrezne meritve in opise. V preglednici 2 (za populacije iz Bohinja) in v preglednici 3 (za populacije iz Štajerske) je prikazan samo del podatkov za petnajst najpomembnejših lastnosti.

Na podlagi vseh zbranih morfoloških podatkov smo izračunali koeficiente podobnosti (Gower, 1971) med posameznimi rastlinami in rezultate grafično predstavili s tridimenzionalnim grafom (Sl. 2). Kaže, da se populacije iz Bohinja (na sl. 2 so označene s temnejšimi (tiskana verzija) oz. črnimi in modrimi znaki (elektronska verzija – dostopna na domači spletni strani <http://aas.bf.uni-lj.si>) na splošno dobro razlikujejo od štajerskih populacij (na sl. 2 so označene s svetlejšimi (tiskana verzija) oz. rdečimi in zelenimi znaki (elektronska verzija)). Izjemi sta le populaciji B1 in B8, ki sta po morfoloških znakih še najbolj podobni štajerskim populacijam. Glede na posamezne morfološke lastnosti B1 odstopa od bohinjskih populacij le po barvi zrnja, ki je ob brezbarvnem perikarpu pogojena z barvo alevrona, saj je edina bohinjka z rumenim zrnjem (Pregl. 2). Vse ostale bohinjske populacije imajo bakreno barvo zrnja, medtem ko imajo štajerske populacije rumeno ali belo zrnje. Populacija B11 je po morfoloških znakih zelo neizenačena, dve rastlini sta blizu oz. med štajerskimi populacijami, vse ostale rastline pa so razpršene med bohinjskimi populacijami (Sl. 2).

Na podlagi 21 kvantitativnih morfoloških lastnosti, od populacij iz Bohinja najbolj odstopata populaciji B9 in B4; ostale tvorijo dve večji skupini: prvo skupino populacije B1, B2, B5 in B12, drugo pa B3, B6, B7, B8 in B11 (Sl. 3). Štajerske populacije se med seboj razlikujejo predvsem glede na prvo in tretjo komponento (Prin1 in Prin3). Zelo podobne so si le Š10 in Š11 ter Š12 in Š13. Sicer pa se obe skupini populacij med sabo razlikujeta predvsem glede na drugo komponento.

Preglednica 2: Nekatere morfološke lastnosti enajstih populacij koruze iz Bohinja (4.1.4 – višina prve stranske metlice, 4.1.5 – višina vrhnjega storža, 4.1.7 – število listov nad storžem, 4.1.13 – % primarni (% sekundarni) tip metlice, 4.2.4 – število vrst zrnja, 6.1.1 – skupno število listov, 6.1.2 – dolžina lista ob storžu, 6.1.3 – širina lista ob storžu, 6.1.8 – dolžina metlice, 6.2.1 – število storžev na rastlino, 6.2.2 – dolžina storža, 6.2.4 – premer storža, 6.2.10 – oblika storža, 4.3.2 – barva zrna, 6.3.6 – barva alevrona).

Table 2: Some of the morphological traits of eleven maize populations from Bohinj (4.1.4 – Plant height, 4.1.5 – Uppermost ear height, 4.1.7 – No. of leaves above the uppermost ear-including ear leaf, 4.1.13 – % primary (% secondary) tassel type, 4.2.4 – No. of kernel rows, 6.1.1 – No. of leaves per plant, 6.1.2 – Leaf length, 6.1.3 – Leaf width, 6.1.8 – Tassel length, 6.2.1 – No. of ears per plant, 6.2.2 – Ear length, 6.2.4 – Ear diameter, 6.2.10 – Shape of ear, 4.3.2 – Kernel color, 6.3.6 – Aleurone color).

IPGRI	4.1.4	4.1.5	4.1.7	4.1.13	4.2.4	6.1.1	6.1.2	6.1.3	6.1.8	6.2.1	6.2.2	6.2.4	6.2.10 ^a	4.3.2 ^b	6.3.6 ^c
B1	163 ±16	60,1 ±8,8	5,7 ±0,8	0,28 (0,72)	14,4 ±2,3	8,6 ±1,2	78,4 ±6,3	9,4 ±0,9	39,1 ±4,3	1,3 ±0,5	17,1 ±1,4	4,4 ±0,2	2	2	1
B2	184 ±14	71,8 ±8,9	5,4 ±0,5	0,65 (0,35)	12,9 ±1,5	8,5 ±0,8	89,7 ±7,3	8,9 ±0,9	48,6 ±9,0	1,0 ±0,0	17,0 ±2,2	4,3 ±0,4	2-1	5	2
B3	186 ±12	72,2 ±14,1	6,5 ±0,9	0,1 (0,9)	12,7 ±1,7	9,6 ±1,2	85,3 ±5,5	8,6 ±0,8	44,6 ±2,9	1,2 ±0,4	15,8 ±1,6	4,4 ±0,2	1	5	2
B4	205 ±15	81,2 ±13,9	6,1 ±0,6	0,0 (1,0)	12,3 ±1,2	9,7 ±0,9	82,9 ±6,6	8,7 ±1,1	42,0 ±4,6	1,2 ±0,4	16,8 ±1,6	4,4 ±0,3	2-3-1	5-9	2
B5	171 ±15	55,4 ±13,6	6,7 ±0,7	0,84 (0,16)	10,7 ±1,2	10,0 ±0,9	77,6 ±5,3	8,8 ±0,8	39,5 ±5,2	1,1 ±0,2	18,5 ±2,8	4,0 ±0,4	2-1	5	2
B6	188 ±11	71,8 ±10,9	5,8 ±0,9	0,65 (0,35)	12,4 ±1,7	9,2 ±0,9	81,3 ±5,7	8,8 ±0,8	42,9 ±7,1	1,0 ±0,0	17,1 ±2,2	4,5 ±0,3	1-2	5	2
B7	185 ±13	59,4 ±11,3	6,9 ±0,6	0,25 (0,75)	11,8 ±1,3	9,4 ±0,8	91,7 ±5,2	9,0 ±0,9	43,0 ±4,0	1,0 ±0,0	18,2 ±1,6	4,3 ±0,2	1-2	5	2
B8	181 ±16	66,4 ±9,1	6,3 ±0,6	0,05 (0,95)	11,4 ±1,5	8,9 ±0,8	86,0 ±5,6	9,7 ±0,9	43,3 ±7,9	1,0 ±0,0	19,9 ±2,3	4,2 ±0,3	2-1	5-2-6	1
B9	138 ±19	47,0 ±10,3	5,6 ±0,8	0,42 (0,58)	11,9 ±1,9	8,7 ±1,1	71,9 ±6,3	9,6 ±0,8	37,6 ±5,1	1,4 ±0,5	14,8 ±2,3	3,9 ±0,4	1	3-5	2
B11	178 ±18	61,9 ±14,2	6,3 ±1,1	0,5 (0,5)	12,6 ±2,2	9,1 ±1,1	83,5 ±6,7	9,1 ±0,9	42,5 ±5,8	1,1 ±0,3	19,9 ±2,2	4,1 ±0,3	1-2	5-2-3	2-1
B12	136 ±18	39,4 ±6,2	6,1 ±0,9	0,29 (0,71)	12,1 ±1,4	8,5 ±0,9	80,2 ±7,3	9,3 ±1,0	39,9 ±6,2	1,4 ±0,5	16,8 ±2,1	4,4 ±0,3	2	5	2

a – 1 – valjasta (cylindrical), 2 – valjasto-stožčasta (cylindrical-conical), 3 – stožčasta (conical), 4 – okrogla (round).

b – 1 – bela (white), 2 – rumena (yellow), 3 – vijolična (purple), 4 – pisana (variegated), 5 – rjava (brown), 6 – oranžna (orange), 7 – lisasta (mottled), 8 – bela kapica (white cap), 9 – rdeča (red).

c – 1 – brezbarvna (colourless), 2 – bronasta (bronze), 3 – rdeča (red), 4 – vijolična (purple), 5 – druga (other).

Preglednica 3: Nekatero morfološke lastnosti štirinajstih populacij koruze iz okolice Vojnika in Laškega (Š1-Š14) (4.1.4 – višina prve stranske metlice, 4.1.5 – višina vrhnjega storža 4.1.7 – število listov nad storžem, 4.1.13 – % primarni (% sekundarni) tip metlice, 4.2.4 – število vrst zrnja, 6.1.1 – skupno število listov, 6.1.2 – dolžina lista ob storžu, 6.1.3 – širina lista ob storžu, 6.1.8 – dolžina metlice, 6.2.1 – število storžev na rastlino, 6.2.2 – dolžina storža, 6.2.4 – premer storža, 6.2.10 – oblika storža, 4.3.2 – barva zrna, 6.3.6 – barva alevrona).

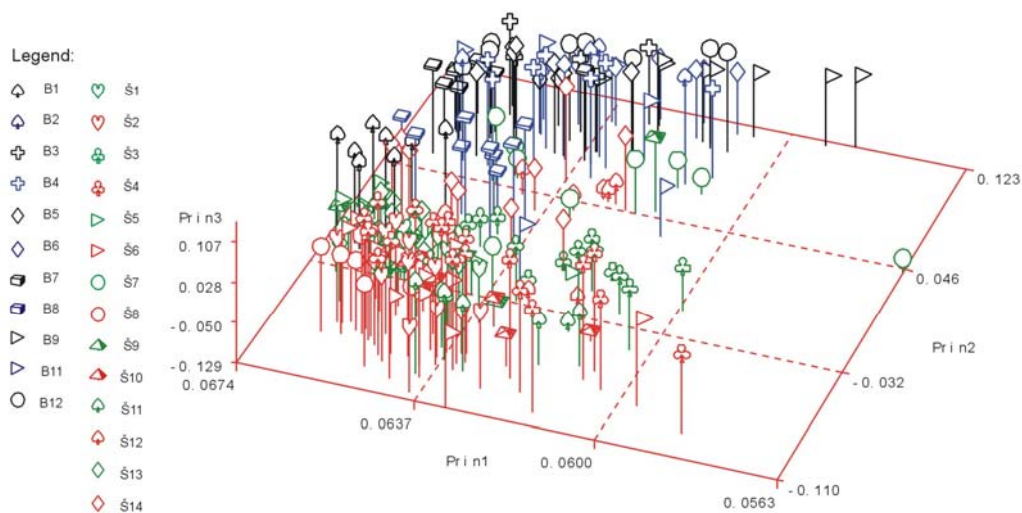
Table 3: Some of the morphological traits of fourteen maize populations from Vojnik and Laško surroundings (4.1.4 – Plant height, 4.1.5 – Uppermost ear height, 4.1.7 – No. of leaves above the uppermost ear-including ear leaf, 4.1.13 – % primary (% secondary) tassel type, 4.2.4 – No. of kernel rows, 6.1.1 – No. of leaves per plant, 6.1.2 – Leaf length, 6.1.3 – Leaf width, 6.1.8 – Tassel length, 6.2.1 – No. of ears per plant, 6.2.2 – Ear length, 6.2.4 – Ear diameter, 6.2.10 – Shape of ear, 4.3.2 – Kernel color, 6.3.6 – Aleurone color).

IPGRI	4.1.4	4.1.5	4.1.7	4.1.13	4.2.4	6.1.1	6.1.2	6.1.3	6.1.8	6.2.1	6.2.2	6.2.4	6.2.10 ^a	4.3.2 ^b	6.3.6 ^c
Š1	204 ±25	82,3 ±17,0	6,3 ±0,9	0,11 (0,89)	12,4 ±1,2	10,0 ±1,4	90,2 ±8,8	10,0 ±0,8	43,1 ±5,5	1,6 ±0,5	20,2 ±2,1	4,3 ±0,4	2-1	2	1
Š2	192 ±21	73,9 ±17,1	6,1 ±1,0	0,05 (0,95)	11,2 ±1,8	9,8 ±1,6	93,1 ±6,5	9,8 ±1,1	43,1 ±6,1	1,4 ±0,5	18,4 ±2,9	4,4 ±0,2	2-1	1	1
Š3	189 ±22	59,4 ±16,1	5,8 ±0,9	0,05 (0,95)	8,1 ±0,8	9,1 ±1,0	84,1 ±8,3	8,9 ±0,9	35,4 ±9,4	1,2 ±0,4	20,9 ±2,6	3,7 ±0,4	2-1	2	1
Š4	200 ±17	83,1 ±15,1	7,0 ±1,1	0,0 (1,0)	12,8 ±1,5	10,7 ±1,3	95,7 ±5,1	10,3 ±1,3	45,0 ±7,7	1,1 ±0,3	20,5 ±2,1	4,5 ±0,4	2	1	1
Š5	184 ±29	70,0 ±20,3	6,3 ±0,9	0,0 (1,0)	8,8 ±1,2	9,3 ±1,4	81,3 ±11,1	10,5 ±1,2	48,6 ±25,1	1,3 ±0,4	22,2 ±2,4	3,8 ±0,3	1-2	2-6	1
Š6	190 ±17	82,9 ±16,1	6,7 ±0,9	0,1 (0,9)	13,0 ±1,2	10,2 ±1,6	86,4 ±6,9	9,2 ±0,9	40,7 ±5,0	1,3 ±0,5	19,3 ±3,4	4,7 ±0,4	2	1-2	1
Š7	193 ±22	63,7 ±15,1	6,2 ±0,8	0,05 (0,95)	8,6 ±1,1	9,4 ±2,8	93,1 ±9,4	11,0 ±1,3	46,5 ±7,7	1,4 ±1,1	20,4 ±3,1	4,2 ±0,3	1-2	6-2	2-1
Š8	202 ±14	72,0 ±15,1	6,3 ±0,7	0,05 (0,95)	10,8 ±1,5	9,3 ±0,9	87,4 ±8,0	9,5 ±0,9	42,7 ±5,8	1,2 ±0,4	19,2 ±2,5	4,2 ±0,3	2-1	1-2	1
Š9	211 ±14	67,8 ±10,3	6,8 ±0,8	0,05 (0,95)	8,7 ±1,3	9,9 ±1,0	86,5 ±12,7	9,2 ±1,1	38,7 ±6,9	1,3 ±0,4	18,9 ±3,1	4,2 ±0,3	1-2	2	1
Š10	180 ±19	59,8 ±14,5	6,2 ±0,5	0,05 (0,95)	8,0 ±0,0	9,2 ±0,9	84,6 ±8,2	9,8 ±1,3	38,9 ±5,0	1,1 ±0,3	15,5 ±2,4	3,9 ±0,3	1-2	1	1
Š11	188 ±20	71,3 ±14,9	5,9 ±0,7	0,11 (0,89)	8,6 ±1,2	9,2 ±1,2	84,7 ±6,4	10,0 ±1,0	35,4 ±6,6	1,4 ±0,5	18,5 ±3,2	4,1 ±0,3	2-1	1-2	1
Š12	200 ±19	66,0 ±12,7	6,4 ±1,0	0,15 (0,85)	8,0 ±0,6	10,1 ±1,5	85,1 ±20,6	9,9 ±1,0	41,2 ±7,7	1,1 ±0,2	18,2 ±3,2	4,1 ±0,4	1	2-6	1-2
Š13	202 ±18	71,3 ±15,5	6,4 ±0,8	0,0 (1,0)	8,4 ±1,1	9,8 ±1,2	88,6 ±6,5	8,9 ±0,7	41,9 ±5,0	1,0 ±0,0	18,7 ±3,1	4,2 ±0,2	1-2	2	1
Š14	190 ±16	55,2 ±14,1	6,2 ±0,9	0,0 (1,0)	9,0 ±1,5	9,4 ±0,9	86,6 ±7,3	9,0 ±1,0	36,3 ±7,3	1,1 ±0,3	19,2 ±2,6	4,2 ±0,4	2-3	6	1-2

a – 1 – valjasta (cylindrical), 2 – valjasto-stožčasta (cylindrical-conical), 3 – stožčasta (conical), 4 – okrogla (round).

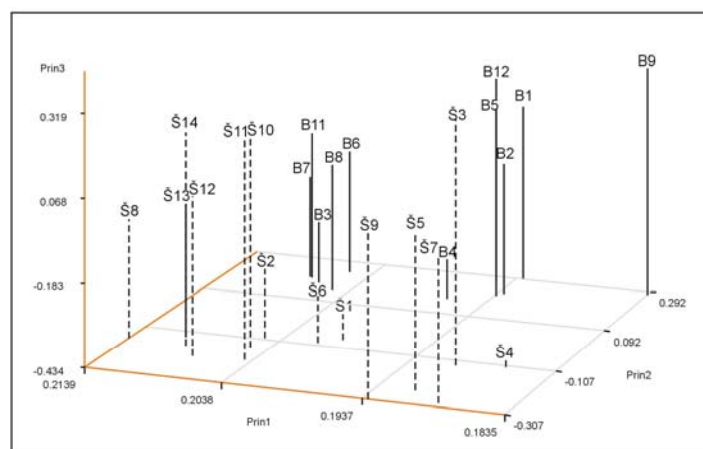
b – 1 – bela (white), 2 – rumena (yellow), 3 – vijolična (purple), 4 – pisana (variegated), 5 – rjava (brown), 6 – oranžna (orange), 7 – lisasta (mottled), 8 – bela kapica (white cap), 9 – rdeča (red).

c – 1 – brezbarvna (colourless), 2 – bronasta (bronze), 3 – rdeča (red), 4 – vijolična (purple), 5 – druga (other).



Slika 2: Odnosi med rastlinami iz 25 slovenskih populacij koruze na podlagi 35 morfoloških lastnosti.

Figure 2: Relationship among plants from 25 Slovenian maize populations based on 35 morphological traits.

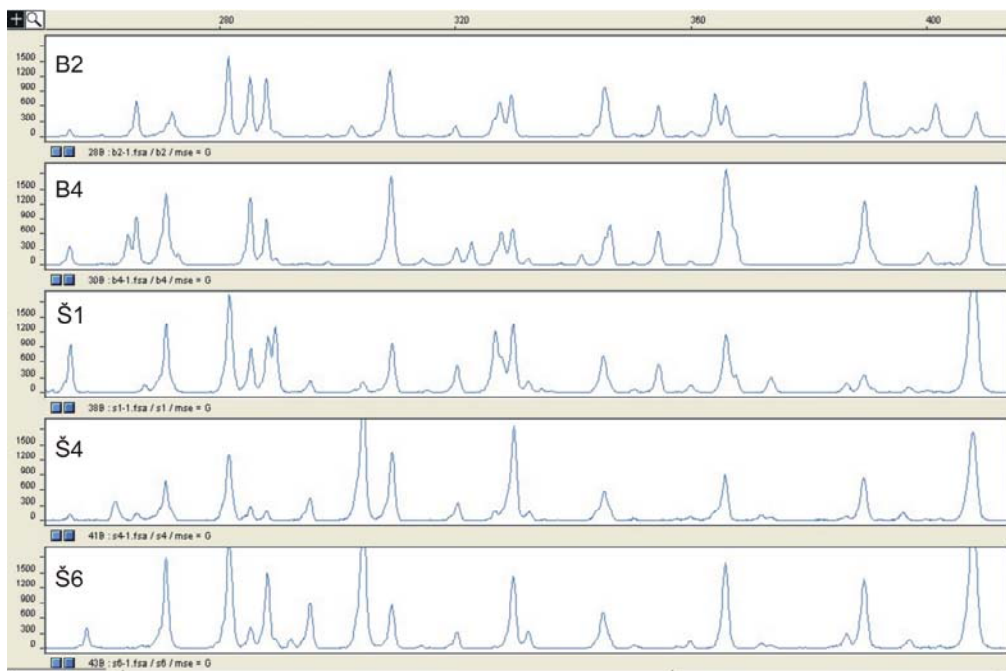


Slika 3: Odnosi med populacijami koruze na podlagi 21 kvantitativnih morfoloških lastnosti.

Figure 3: Relationship among Slovene maize populations based on 21 quantitative morphological traits.

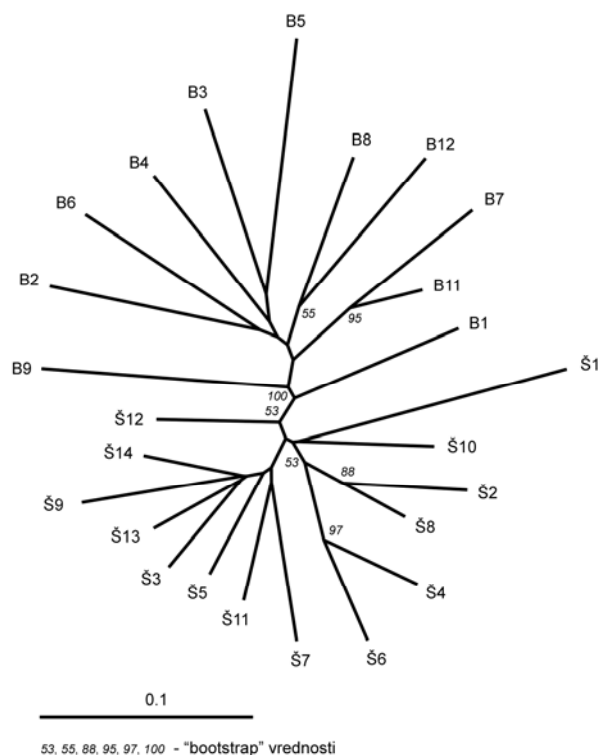
Genetski opis

Populacije koruze smo genetsko opisali s 161 *Hbr* markerji (Sl. 4). Vsaka populacija je imela različno kombinacijo *Hbr* elementov. Razen štirih *Hbr* elementov, smo vse ostale zasledili v več kot eni populaciji. Precejšnje razlike med populacijami so bile tudi v pogostosti posameznih *Hbr* elementov.



Slika 4: Kratek odsek z DNK profili petih populacij. *Hbr* elementi z različnih lokacij v genomu se ločijo glede na različno dolžino PCR produktov.

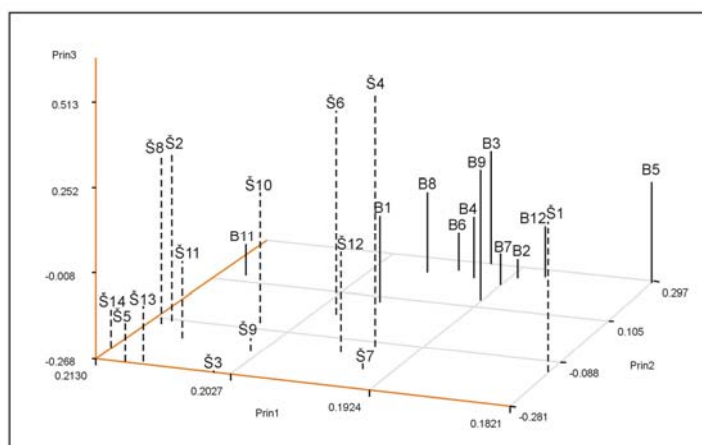
Figure 4: A short portion of DNA profiles of five populations. *Hbr* elements from different genome locations differed in PCR products length.



Slika 5: Odnosi med populacijami koruze na podlagi 161 *Hbr* markerjev predstavljeni z NJ drevesom.

Figure 5: NJ tree representing relationship among Slovene maize populations (based on 161 *Hbr* markers).

Kot je bilo za pričakovati že na podlagi morfoloških podatkov, se tudi na podlagi genetskih podatkov populacije iz Bohinja jasno ločijo od štajerskih populacij (Sl. 5, Sl. 6). Izmed štajerskih populacij genetsko še najbolj odstopa populacija Š1. Relativno blizu, glede na ostale populacije, sta si populaciji Š4 in Š6 ter populaciji Š2 in Š8. Blizu so si tudi populacije Š3, Š5, Š9, Š11, Š13 in Š14. Med bohinjskimi populacijami najbolj odstopa populacija B5 (Sl. 5, Sl. 6). Glede na prisotnost in pogostost istih *Hbr* elementov sta si najbolj podobni populaciji B7 in B11 (Sl. 5). Ti dve populaciji sta si podobni tudi po povprečnih kvantitativnih morfoloških lastnostih (Sl. 3). S PCA analizo 161 *Hbr* markerjev (Sl. 6) se B7 uvršča v dokaj homogeno skupino, ki jo tvori večina bohinjskih populacij, medtem ko od vseh bohinjških populacij nekoliko bolj odstopata B5 in B11.



Slika 6: , Odnosi med populacijami korusa na podlagi 161 *Hbr* markerjev.
Figure 6: PCA analysis representing relationship among Slovenian maize populations based on 161 *Hbr* markers.

Čeprav je tridimenzionalna razporeditev posameznih populacij s PCA analizo, prikazana na podlagi morfoloških lastnosti (Sl. 3) nekoliko drugačna od razporeditve na podlagi 161 *Hbr* markerjev (Sl. 6), rezultati obeh analiz kažejo na to, da se populacije iz Bohinja jasno razlikujejo od populacij iz Štajerske. Glede na vse analize, še posebej na prisotnost/odsotnost *Hbr* elementov na določeni lokaciji ter pogostnost pojavljanja posameznih *Hbr* elementov, lahko kljub nekaterim podobnostim med posameznimi populacijami trdimo, da gre za različne populacije, čeprav nekatere izhajajo iz lokacij, ki so si geografsko zelo blizu. Populacije B4, B5 in B11 ter Š1, Š2 in Š4 izhajajo iz enega kraja v Bohinju oz. na Štajerskem, a so od različnih pridelovalcev. Torej so posamezni pridelovalci s svojim vzdrževanjem in ponovno setvijo lastnega semena uspeli obdržati karakteristične lastnosti svoje populacije, s tem da so preprečili nekontrolirano skrižanje z drugimi populacijami.

VIRI

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Agris category code: F60, Q04, P01

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Morphometrical and biochemical characteristics of red grape varieties (*Vitis vinifera* L.) from collection vineyard Ampelografski vrt

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ABSTRACT

Diversity of vine varieties is confirmed by different morphological and biochemical characteristics of each variety, especially in bunch and berry properties. The evaluation of mentioned characteristics supplements the chemotaxonomical classification and preservation of local and less known vine varieties. Morphometrical and biochemical characteristics were determined on grape berries of 14 different vine varieties, called 'Barbera', 'Merlot', 'Cabernet sauvignon', 'Syrah', 'Refošk', 'Sladki teran', 'Teran Istra', 'Pokalca', 'Plavina', 'Plovdina', 'Sladkočrn', 'Tinta Pinheira', 'Vranac' in 'Zweigelt', grown in Ampelographic vineyard. Morphological parameters were determined by morphometry, O.I.V. descriptors number codes 220, 221, 503 and with colorimeter, but biochemical parameters with several carbohydrates by HPLC and total acidity with titration. The obtained results confirmed some similarity among selected varieties, and multivariable analyses according to determined parameters group the varieties into 4 groups; in group I varieties 'Barbera Bovcon', 'Pokalca' and 'Barbera standard'; group II varieties 'Refošk', 'Syrah' and 'Teran Istra'; group III varieties 'Cabernet sauvignon', 'Merlot' and 'Zweigelt'; group IV varieties 'Sladkočrn', 'Tinta Pinheira' and 'Vranac'. The variety 'Plovdina' is not included in any group, what confirms its vast variability compared with other varieties taken into account.

Key words: berry, grapevine, quality, colour

IZVLEČEK

MORFOMETRIČNE IN BIOKEMIJSKE LASTNOSTI RDEČIH SORT VINSKE TRTE (*Vitis vinifera* L.) IZ KOLEKCIJSKEGA VINOGRADA AMPELOGRAFSKI VRT

Diverzitetu sort vinske trte (*Vitis vinifera* L.) potrjujejo različne morfološke in biokemijske lastnosti rastlinskih delov, predvsem grozda in jagodah. Z vrednotenjem navedenih parametrov se dopolnjuje kemotaksonomsko klasifikacijo sort in posredno se pripomore k ohranjanju starih in manj znanih sort. Morfološke in biokemijske lastnosti smo določali na jagodah 14-tih sort vinske trte: 'Barbera', 'Merlot', 'Cabernet sauvignon', 'Syrah', 'Refošk', 'Sladki teran', 'Teran Istra', 'Pokalca', 'Plavina', 'Plovdina', 'Sladkočrn', 'Tinta Pinheira', 'Vranac' in 'Zweigelt', ki rastejo v Ampelografskem vrtu. Morfološke lastnosti smo določili po

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metodah ampelometrije, z O.I.V. deskriptorji 220, 221, 503 in kolorimetrom, medtem ko biokemijske z vrednotenjem količine posameznih ogljikovih hidratov s HPLC in skupne kisline s titracijo. Rezultati, se s statistično multivariatno analizo, glede na ovrednotene lastnosti gručirajo v štiri skupine s podobnimi lastnostmi, in sicer; skupina I sorte 'Barbera Bovcon', 'Pokalca' in 'Barbera standard'; skupina II sorte 'Refošk', 'Syrah' in 'Teran Istra'; skupina III sorte 'Cabernet sauvignon', 'Merlot' in 'Zweigelt'; skupina IV sorte 'Sladkočrn', 'Tinta Pinheira' in 'Vranac', kar kaže na njihovo podobnost. Sorta 'Plovdina' se ne uvršča v nobeno od skupin, kar dokazuje njeno večjo variabilnost od ostalih vzorčenih sort.

Ključne besede: jagoda, vinska trta, kakovost, barva

1 INTRODUCTION

There are around 10 000 different grape varieties and their number has been increasing in recent years, mostly at the expense of hybrids and less known local varieties (Martinez de Toda, 1991). Characterization of varieties has been made more difficult due to divergent morphological characteristics of individual parts of grapevine from different winegrowing areas or due to synonym and homonym use with the same varieties. Apart from these differences, these varieties share numerous similarities that are most frequently established by determining morphological and biochemical characteristics of grape and berries (Galet, 1990; Carreño *et al.*, 1997). Grapevine varieties could be evaluated in a number of ways, with morphological descriptions method (O.I.V. descriptors) and morphometrics being the most common (Galet, 1952; Cabello *et al.*, 1993).

Morphological features of grape and berries are of genotypic nature, although they are significantly influenced by environmental factors (air temperature, precipitation, wind etc.) (Smart and Robinson, 1992). Grape quality is both created and determined by carbohydrates, organic acids and phenolic substances (Winkler *et al.*, 1974; Mullins *et al.*, 1992).

The most important carbohydrates in terms of quantity are glucose, fructose and sucrose as they taken together account for around 95% of total sugar quantity. The most important organic acids, on the other hand, are tartaric acid, malic acid and citric acid that supply must and subsequent wine with their respective freshness. Sucrose is hydrolysed into fructose and glucose in berries, and their resultant concentration is in relation to that of fructose higher in the berries' first phase of growth and maturation. Fructose is on average twice sweeter as compared to glucose, and the ratio of both sugars also depends on the genetic potential of grapevine varieties (Garcia-Romero *et al.*, 1993; Roubelakis-Angelakis, 2001; Clancy, 2002; Kennedy, 2002). Artes-Hdez *et al.* (2004) indicates the ratio between glucose/fructose on average between 0.98 and 1.05. More significance has been recently attached to phenolic substances or what is referred to as phenolic grape maturation. The latter is determined by phenolic substances and acids which are important in terms of quantity as they provide grape with colour and taste, aroma and bouquet (Stafford, 1990; Perez-Magariño in Gonzalez-San Jose, 2005). The very beginnings of grape maturation are accompanied by coloration of berry skin, and their colour intensity increases with maturation. This means that the aim is to attain the optimal coloration of berries when grape

harvesting. Grape coloration depends on its variety (genotype) and environmental factors, mostly ultraviolet radiation, light and heat (Mullins *et al.*, 1992).

By determining and evaluating both the differences and similarities of morphological parts of red grape varieties grown in collection vineyard Ampelografski vrt, an attempt has been made to upgrade the database on morphological and biochemical potential of individual variety as it is reasonable to maintain genetic variability in Slovenia and in doing so also retain specific characteristics of some older and less known varieties.

2 MATERIALS AND METHODS

2.1 LOCATION AND DESCRIPTION OF THE VINEYARD

The study was carried out on 14 red, old and also on the globally known grapevine varieties (*Vitis vinifera* L.) cultivated in the Ampelographic garden of Kromberk near Nova Gorica. The selected varieties include 'Barbera', 'Merlot', 'Cabernet Sauvignon', 'Syrah', 'Refošk', 'Sladki Teran', 'Teran Istra', 'Pokalca', 'Plavina', 'Plovdina', 'Sladkočrn', 'Tinta Pinheira', 'Vranac' and 'Zweigelt'. Grape production is a part of integrated production.

2.2 SAMPLING AND MEASUREMENTS OF MORPHOLOGICAL PROPERTIES OF BERRIES

2.2.1 Cluster Sampling

Berries and entire grapes were sampled in the morning. A random method was used for the selection of both clusters on grapevine, and berries on cluster. The average weight of the sample amounted to 1500 g per variety. Berries and grapes were immediately transferred to the laboratory, stored in PE bags and kept in the refrigerator at $-20\text{ }^{\circ}\text{C}$ until extraction and sample preparation for measurements and analysis.

2.2.2 Determining Morphometrical Properties of Berries

Similarities and differences between varieties were determined by way of the morphometry of berries and by way of the method employing O.I.V. descriptors. Berry samples were weighted, while length, width and size as stated in O.I.V. descriptors 220, 221 and 503 were also defined for individual berries. O.I.V. descriptor 220 sets out 5 berry sizes, i.e. very small, small, medium, large and very large berries. O.I.V. descriptor 503 determines single berry weight, with 1 g being considered as a very small weight, approximately 2 g as a small weight, approximately 4 g as medium, approximately 8 g as large and above 12 g as a very large berry weight. Berry length was determined in line with O.I.V. descriptor 221, i.e. very short (up to 10 mm), short (10-17 mm), medium (17-24 mm), long (24-31 mm) and very long (above 31 mm) berries (O.I.V. descriptors, 1983). The data is expressed in mean millimetres and milligrams by a standard error.

2.2.3 Determining Skin Colour of the Berry

Skin colour of the berry was determined by using O.I.V. descriptor 225 classifying grape colours into 7 groups, i.e. green-yellow, rose, red, red-grey, dark red-violet, blue-black and red-black (O.I.V. descriptors, 1983). The visual assessment of berry colour is performed by using and comparing the listed varieties in the descriptor.

A more precise method was applied for the determination of skin colour of the berry, and it involved the colorimeter Minolta CR-300 Chroma (Minolta Co; Osaka, Japan) with L^* , a^* and b^* values as colour space coordinates (CIE $L^*a^*b^*$). The colorimeter is first calibrated using a white calibration plate. L^* values represent a black and white scale (0=black; 100=white) or relative 'darkness' of the colour. The value is low in cases of dark shades and high for light

colour shades. A^* and b^* values change within a range between -60 to +60, with a^* being negative for green colour and positive for red, while b^* value is negative for blue and positive for yellow. The tint (H) is calculated on the basis of $\tan^{-1}(b^*/a^*)$ and is expressed in degrees ($^\circ$); 0° = red, 90° = yellow, 180° = green and 270° = blue (Lancaster, 1992; McGuire, 1992).

2.3 EXTRACTION FOR CARBOHYDRATE ANALYSIS USING HPLC

2.3.1 Sample Preparation for HPLC Analysis

Berries and grape juice were prepared to suit the needs of the analysis by employing the method described by Dolenc and Štampar (1997) with minor alterations. Purified deionised water (dd, water; MilliQ purification system, Millipore, France) was used for the extraction of individual carbohydrates. Grape juice was squeezed; 1 mL was pipetted off and diluted with 10 ml of dd water (V/V). The samples were centrifuged for 7 min at room temperature and 4200 rpm. The supernatant was used for analysis, and filtered to pass through a 0.45 μm membrane filter Chromafil A-45/25 (Macherey-Nagel) prior to on-column injection.

2.3.2 Chromatographic Conditions of Carbohydrate Analysis

HPLC system: Thermo Separation Products (TSP) - binary pump P2000 (Spectra system), automatic sample feeder AS 1000 (Spectra System), degassifier: A-ACT™ Your Research; mobile phase: bidistilled water, flow rate of the mobile phase: 0,6 mL/min, injected sample volume: 20 μl ; analytical column: Phenomenex (Rezex RCM-Monosaccharid Ca^+ ; 300 x 7.80 mm), working temperature of the column: 65 $^\circ\text{C}$, detector: Shodex RI-71, time for sample analysis: 45 min; software: ChromQuest.

The concentration of soluble carbohydrates was calculated by using external standards. Glucose, fructose and sucrose standards were employed (Fluka).

2.3.3 Determining Total Sugar Contents and Titration Acid

Total sugar contents were determined by way of the manual refractometer (Atago, Kuebler, 30-130 $^\circ\text{Öechsle}$). A drop of grape juice is dropped on the optical glass, and the sugar amount in the sample is read by facing the glass towards the light. The data was given in $^\circ\text{Öe}$. Titration acid was determined by employing a traditional method also stated in Šikovec (1993). 12.5 mL of grape juice was pipetted off to the conical flask and some drops of the dye indicator were added. 0.1 M NaOH was used for titration. The used mL of lye was multiplied by a factor 0.75 to obtain the amount (mL) of titration acid in L of grape juice. The results are expressed in g per L.

2.4 STATISTICAL DATA ANALYSIS

The data was evaluated with the help of the programme Statgraphics plus 4.0 software and the multivariate analysis with clustering was performed. The results are given as means and standard errors.

3 RESULTS AND DISCUSSION

3.1 MORPHOMETRICAL PROPERTIES OF BERRIES

3.1.1 Berry Size and Weight

Table 1 compiles data on more important morphometrical properties of berries, such as average berry length (height), width and average weight. It has been observed that average berry length of the selected varieties is within the range from 12.4 to 18.1

mm. The shortest berries were found in 'Zweigelt', 'Merlot' and 'Cabernet Sauvignon' varieties, whereas the longest berries were determined for 'Barbera', 'Vranac' and 'Plavina' varieties. When comparing berry width, it was established that 'Vranac', 'Plavina' and 'Refošk' varieties have the widest berries, and 'Zweigelt', 'Sladki Teran' and 'Merlot' varieties the narrowest. The length/width ratio revealed that 'Barbera standard' variety has the maximum ratio, which points to the oval shape of the berry. The oval shape of the berry is also assigned to 'Barbera Bovcon', 'Plovdina', 'Pokalca', 'Refošk', 'Sladki Teran', 'Syrah' and 'Vranac' varieties (Table 1).

Table 1: Physical characteristics of grape berries of different red varieties. Means and standard errors are presented.

Variety	Length (L) (mm)	Weight (W) (mm)	Size (O.I.V. 220)	Ratio L/W	Berry weight (mg)
Barbera Bovcon	16.7 ± 0.6	15.1 ± 0.5	254.5 ± 14.8	1.1	2339.2 ± 101.9
Barbera standard	18.1 ± 0.3	15.4 ± 0.3	279.4 ± 9.4	1.2	2590.8 ± 42.5
Cabernet sauvignon	13.7 ± 0.3	13.2 ± 0.3	183.1 ± 8.5	1.0	1567.6 ± 33.6
Merlot	13.2 ± 0.4	13.1 ± 0.5	174.5 ± 11.3	1.0	1535.2 ± 44.5
Plavina	17.4 ± 0.5	16.5 ± 0.4	290.6 ± 15.3	1.0	2638.6 ± 52.5
Plovdina	17.0 ± 0.3	15.5 ± 0.4	264.4 ± 10.7	1.1	2232.2 ± 53.5
Pokalca	17.2 ± 0.6	15.2 ± 0.4	264.4 ± 15.8	1.1	2347.0 ± 63.7
Refošk	16.9 ± 0.6	15.8 ± 0.6	270.0 ± 19.3	1.1	2511.2 ± 56.6
Sladki teran	14.3 ± 0.4	13.0 ± 0.4	187.6 ± 11.5	1.1	1512.0 ± 38.1
Sladkočrn	15.3 ± 0.5	15.1 ± 0.5	234.7 ± 15.7	1.0	1129.3 ± 24.5
Syrah	15.5 ± 0.3	13.6 ± 0.3	212.8 ± 8.1	1.1	1719.0 ± 17.2
Teran Istra	14.1 ± 0.3	13.5 ± 0.3	192.7 ± 9.0	1.0	1734.6 ± 53.9
Tinta Pinheira	14.0 ± 0.4	13.8 ± 0.3	195.5 ± 9.5	1.0	1873.7 ± 194.6
Vranac	17.8 ± 0.6	16.7 ± 0.8	301.6 ± 22.5	1.1	3280.0 ± 69.3
Zweigelt	12.4 ± 0.5	12.1 ± 0.5	153.3 ± 11.8	1.0	1133.6 ± 33.5

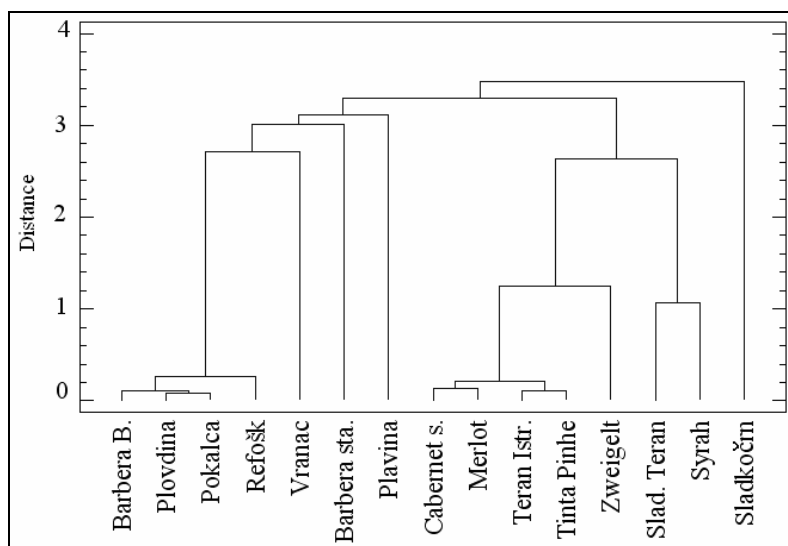


Figure 1: Dendrogram according to berry size, form and weight of selected grape varieties. Taking into consideration the morphometric characteristics of berries as shown in Table 1, some similarities have been established between 'Barbera Bovcon', 'Plovdina', 'Pokalca' and 'Refošk' varieties (group 1), between 'Cabernet sauvignon', 'Merlot', 'Teran Istra', 'Tinta Pinheira' and 'Zweigelt' varieties (group 2), and between 'Sladki teran' and 'Syrah' varieties (group 3). All remaining varieties individually form their own groups (Figure 1).

Measurements have demonstrated that 'Vranac', 'Plavina' and 'Barbera standard' varieties have the heaviest berries, while 'Zweigelt', 'Sladkočrn' and 'Sladki teran' have the lightest berries. 'Barbera Bovcon' and 'Tinta varieties displayed the highest variability of average berry weight which in turn indicates unequal width of berries on grape.'

3.1.2 Colour of Berries

There are a number of methods in place for determining berry colour, with O.I.V. descriptor method and colour evaluation with different indexes based on colorimeter colour value being the most frequent. Table 2 illustrates the results of evaluating both methods.

O.I.V. descriptor 225 classifies varieties according to berry skin colour into 7 groups: group '1' greenish yellow, '2' reddish 'rose', '3' red, '4' red-grey hue, '5' dark red-purple, '6' blue-black and '7' red-black.

Table 2: The average O.I.V., L* and hue angle (h) values of berry skin from red grape varieties. Means and standard errors are presented.

Variety	O.I.V. value	L* value	Hue angle (°)
Barbera Bovcon	4	29.1 ± 0.3	60.9 ± 13.6
Barbera standard	5 – 6	28.4 ± 0.3	69.3 ± 36.1
Cabernet sauvignon	5	30.9 ± 0.4	73.6 ± 31.7
Merlot	5	29.7 ± 0.3	78.0 ± 38.1
Plavina	/	/	/
Plovdina	1 – 2	32.6 ± 0.5	26.4 ± 4.2
Pokalca	4 – 5	30.1 ± 0.3	70.7 ± 26.6
Refošk	5	28.6 ± 0.4	69.1 ± 14.5
Sladki teran	/	/	/
Sladkočrn	5	32.9 ± 0.4	79.9 ± 19.8
Syrah	5	28.9 ± 0.3	64.8 ± 14.1
Teran Istra	6	30.8 ± 0.4	80.4 ± 35.1
Tinta Pinheira	4 – 5	29.9 ± 0.3	76.9 ± 26.2
Vranac	6	30.0 ± 0.3	83.1 ± 14.6
Zweigelt	5	30.5 ± 0.3	79.0 ± 22.5

Table 2 provides data on berry skin colour according to varieties. By using O.I.V. descriptor, varieties have been divided into individual groups, which more or less correspond with skin colours of varieties in the descriptor: 'Plovdina', the least coloured red grape variety, has been assigned value between 1 and 2. Most varieties have been classified into class 5, while 'Vranac' and 'Teran Istra' varieties have been ascribed the darkest colour of class 6.

When evaluating L* value, the biggest hues for lightness have been determined for 'Plovdina' and 'Sladkočrn' varieties, while the least value was established for 'Barbera standard', 'Refošk' and 'Syrah', which have been ascribed darker hues and correspond with the visual assessment with O.I.V. descriptors. When comparing hue angles (h (°)), the smallest hue angle was determined with 'Plovdina' variety which is attributable to a poorer berry skin coloration.

As expected, 'Vranac' and 'Teran Istra' varieties display the highest angle in terms of O.I.V. descriptor value and L* value. By taking into consideration all the results of different evaluation methods of berry skin colour, sampled varieties may in terms of their similarities divided into 4 groups. Minor differences in berry skin colour have been established between 'Barbera', 'Refošk' and 'Syrah' varieties (group 1), followed by those between 'Cabernet sauvignon', 'Zweigelt' and 'Merlot' varieties and 'Pokalca' and 'Merlot' varieties as well as between 'Teran Istra' and 'Vranac' varieties (all in group 2), 'Sladkočrn' (group 3) and 'Plovdina' (group 4) (Figure 2).

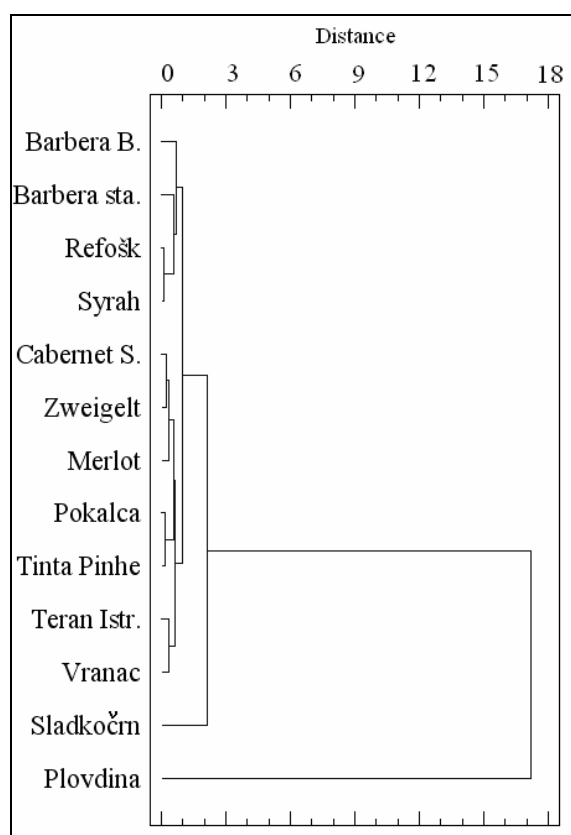


Figure 2: Dendrogram according to colour of berries of selected grape varieties.

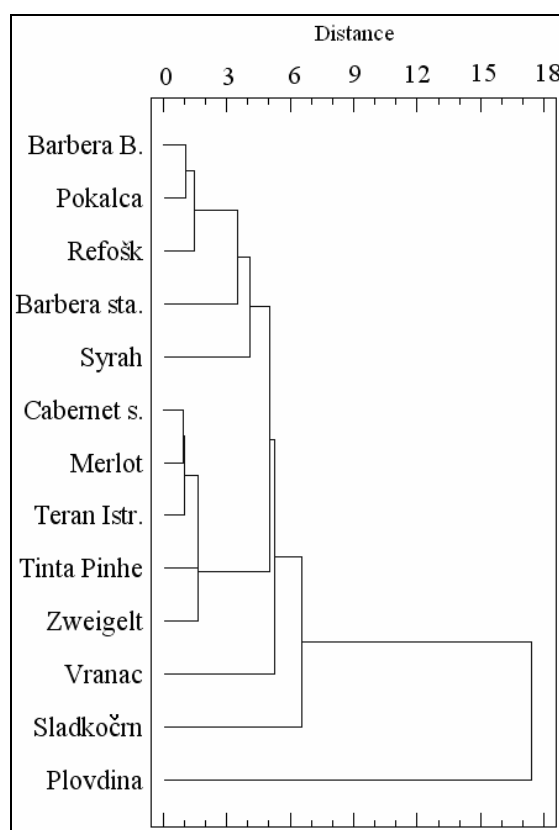


Figure 3: Dendrogram according to berry morphological characteristics of selected grape varieties.

Figure 3 illustrates the differences among varieties in all evaluated morphological characteristics of grape berry. It has been established that samples varieties can be combined at least into 2 groups with some similarities. Similarities in terms of morphological characteristics can be ascribed to 'Barbera Bovcon', 'Pokalca' and 'Refošk' varieties, as well as 'Cabernet sauvignon', 'Merlot', 'Teran Istra', 'Tinta Pinheira' and 'Zweigelt' varieties. All remaining varieties with major differences are not classified into these 2 groups.

3.2 BIOCHEMICAL CHARACTERISTICS OF GRAPE BERRIES

3.2.1 Carbohydrates and total acid contents

Sugar content which is determined with manual refractometer remains to be an increasingly more important indicator of grape quality and consequently of grape

price. Sugar content in grape is also important in determining the time of technological maturity and grape-harvesting.

Figure 4 illustrates average sugar content in tests according to varieties. The smallest sugar content (Öe) has been determined in 'Plovdina', 'Tinta Pinheira' and 'Sladkočrn' varieties, while the highest has been established in 'Zweigelt', 'Merlot', 'Barbera Bovcon' and 'Cabernet sauvignon'.

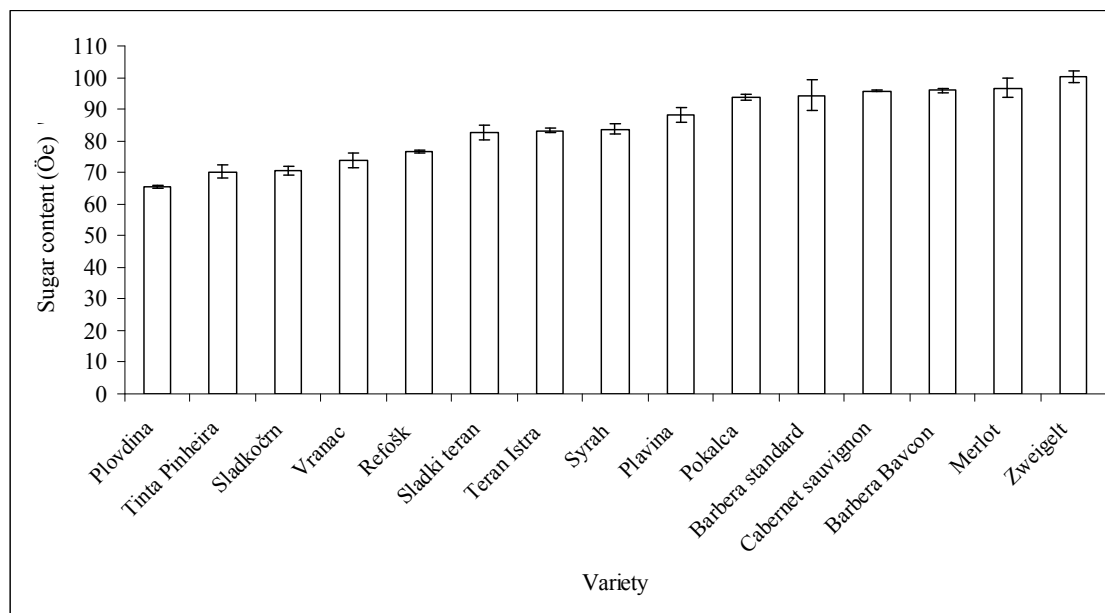


Figure 4: Total sugar content (Öe) with standard errors among different red wine varieties.

Table 3 illustrate average content of individual carbohydrate in grape juice. Major sucrose contents have been determined in 'Barbera standard', 'Teran Istra', 'Syrah' and 'Refošk' varieties, while all remaining varieties displayed 2 to 4 times lower sucrose contents. The biggest glucose contents has been established in 'Merlot' and 'Zweigelt' varieties, while the lowest content has been determined in 'Plovdina' and 'Sladkočrn' varieties. Similar contents for these varieties in glucose have been also determined in fructose content. 'Merlot' and 'Zweigelt' varieties have had the biggest content, while 'Plovdina', 'Sladkočrn' and 'Tinta Pinheira' have shown the lowest content of carbohydrates (Table 3).

Table 3: Selected carbohydrate contents in grapes of different red vine varieties. Means and standard errors are presented.

Variety	Sucrose	Glucose (G)	Fructose (F)	Total	Ratio G/F
Barbera Bovcon	3.6 ± 0.2	84.4 ± 1.8	80.2 ± 1.4	168.2 ± 2.9	1.0
Barbera standard	16.8 ± 1.0	81.5 ± 4.8	78.7 ± 4.3	177.0 ± 8.8	1.0
Cabernet sauvignon	3.2 ± 0.3	78.7 ± 0.8	75.8 ± 0.7	157.7 ± 1.7	1.0
Merlot	10.0 ± 2.9	89.9 ± 4.8	86.3 ± 5.4	186.2 ± 11.5	1.0
Plavina	3.4 ± 0.4	73.7 ± 3.0	73.9 ± 2.8	151.0 ± 5.8	1.0
Plovdina	3.1 ± 0.2	50.9 ± 0.8	55.3 ± 0.5	109.2 ± 1.4	0.9
Pokalca	6.0 ± 0.2	79.8 ± 1.7	77.6 ± 0.8	163.4 ± 2.3	1.0
Refošk	15.3 ± 0.6	59.5 ± 0.7	62.6 ± 0.6	137.4 ± 1.5	0.9
Sladki teran	4.7 ± 1.8	68.5 ± 2.3	72.4 ± 2.9	145.6 ± 4.2	0.9
Sladkočrn	4.7 ± 0.2	51.4 ± 1.4	56.8 ± 2.2	112.9 ± 3.4	0.9
Syrah	15.9 ± 1.6	67.8 ± 2.0	67.9 ± 1.9	151.7 ± 4.9	1.0
Teran Istra	15.0 ± 1.4	62.8 ± 0.8	70.3 ± 1.1	148.1 ± 2.4	0.9
Tinta Pinheira	3.3 ± 0.3	56.7 ± 2.6	54.8 ± 1.9	114.9 ± 4.8	1.0
Vranac	3.0 ± 0.2	65.3 ± 3.1	62.0 ± 3.0	130.4 ± 6.2	1.0
Zweigelt	7.1 ± 0.4	87.5 ± 1.5	83.9 ± 1.0	178.6 ± 2.2	1.0

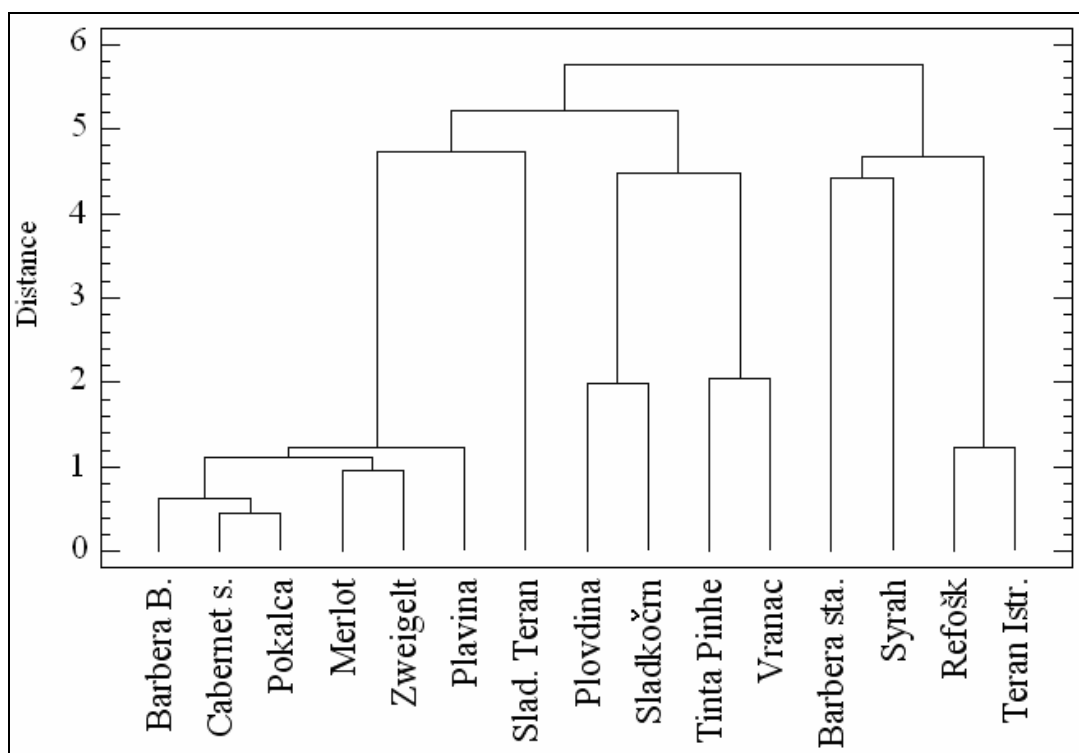


Figure 5: Dendrogram according to sugar content (Öe) and selected carbohydrates in grape juice of grape varieties.

The varieties can be in terms of sugar content and certain carbohydrates content combined into a number of groups. Similarities (figure 5) have been established between 'Barbera Bovcon', 'Cabernet sauvignon', 'Pokalca', 'Merlot', 'Zweigelt' and 'Plavina' (group 1) varieties, as well as by 'Plovdina', 'Sladkočrn', 'Tinta Pinheira' and 'Vranac' (group 2) varieties, and between 'Barbera standard' and 'Syrah' varieties (group 3), and lastly between 'Refošk' and 'Teran Istra' (group 4) varieties.

Besides carbohydrates, organic acids are also important in grape. The lowest content of organic acids has been determined in 'Plovdina' variety, while the highest content

has been established in 'Zweigelt', 'Merlot', 'Barbera Bovcon' and 'Cabernet sauvignon' varieties.

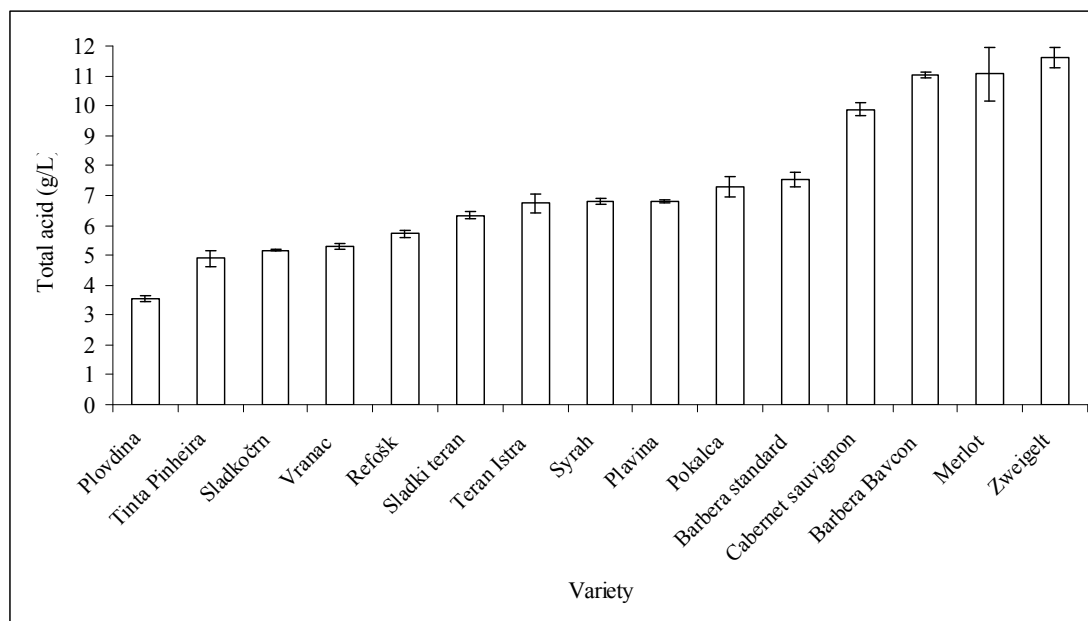


Figure 6: Total acid content (g/L) in grape juice of different red vine varieties. Means and standard errors are presented.

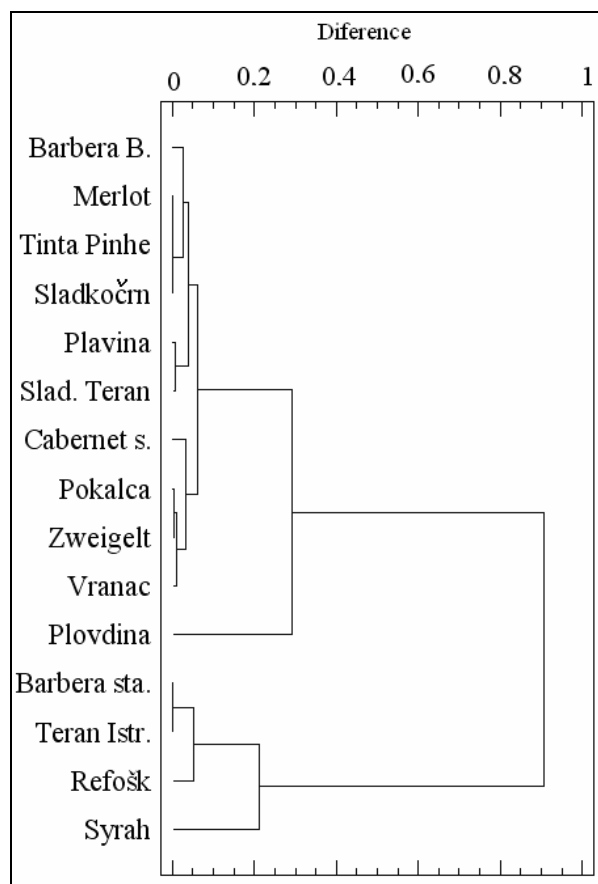


Figure 7: Dendrogram according to total acid (g/L) in grape juice of selected grape varieties.

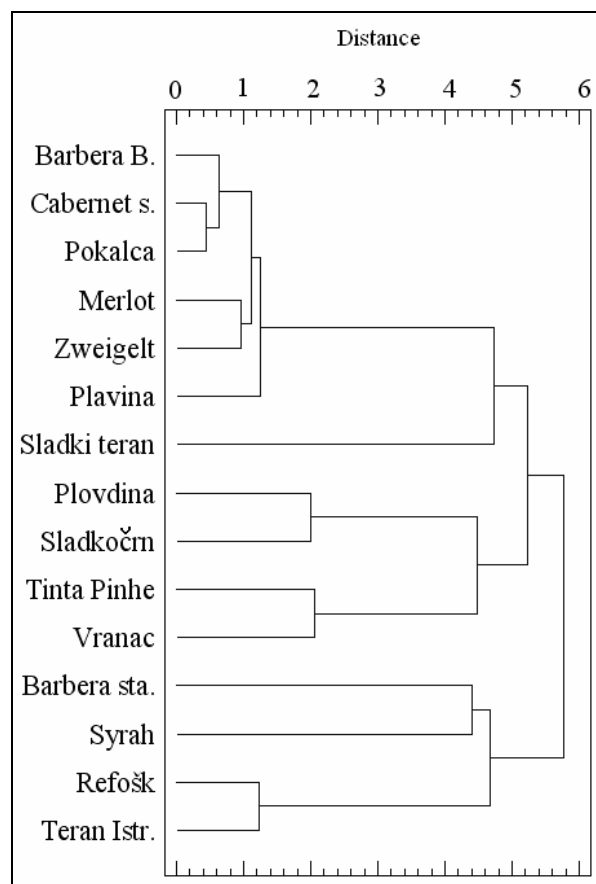


Figure 8: Dendrogram according to biochemical characteristics of grape berries.

When comparing varieties in terms of organic acids content in grape juice, it has been established that the varieties could be divided into 3 groups which display more or less the same acid content. Group 1 includes 'Barbera Bovcon', 'Merlot', 'Tinta Pinheira', 'Sladkočrn', 'Plavina', 'Sladki teran', Cabernet sauvignon', 'Pokalca', 'Zweigelt' and 'Vranac' varieties; group 2 consist of v 'Barbera standard', 'Teran Istra', 'Refošk' and 'Syrah' varieties, while 'Plovdina' makes up its own 3 group.

When comparing figures 7 and 8, it can be concluded that both dendrograms are the same. This means that organic acids make only a minor contribution to the variability of biochemical characteristics of berries, therefore it is suggested that also individual organic acids should be evaluated.

3.3 SIMILARITY OF RED VARIETIES

Figure 9 illustrates the differences among red grape varieties in terms of evaluated morphological and biochemical characteristics of grape berry. The selected varieties have been according to their characteristics divided into the following 4 groups: group I 'Barbera Bovcon', 'Barbera standard' and 'Pokalca'; group II 'Cabernet sauvignon', 'Merlot' and 'Zweigelt'; group III 'Refošk', 'Syrah' and 'Teran Istra', and group IV 'Sladkočrn', 'Tinta Pinheira' and 'Vranac'; 'Plovdina', on the other hand, has not been classified in either of these groups.

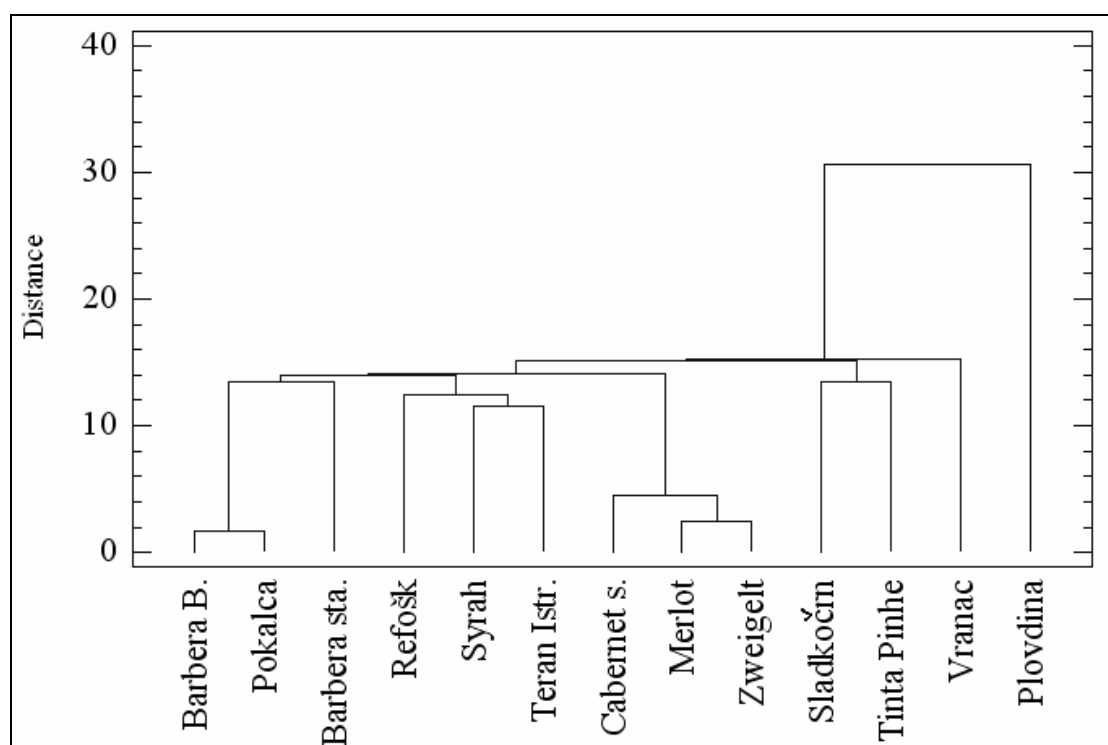


Figure 9: Dendrogram according to morphological and biochemical characteristics of berries of selected grape varieties.

Affinity in terms of morphological characteristics of berries is often connected with similarity of varieties in grape quality. Figure 9 vividly illustrates that the variety types of 'Barbera' are in the same group even though the difference between them is fairly substantial. 'Refošk' and 'Teran Istra' varieties have been ascribed similarity, a fact corroborated by our dendrogram.

4 CONCLUSIONS

Evaluating morphological and biochemical characteristics of grape berries is crucial mainly to chemotaxonomic classification of varieties, and by extension to maintaining variability of variety genotype, not least because the latter mostly differ in grape and berry characteristics. Despite various differences among varieties there are also some similarities which have in turn been corroborated by our results. 14 red grape varieties from the vineyard collection Ampelografski vrt Kromberk have been selected and they have been evaluated for their morphological and biochemical characteristics. Morphologic characteristics have been determined through berry morphometry, O.I.V. descriptor method and colorimeter, while biochemical characteristics have been pinpointed by evaluating carbohydrates and organic acids.

The varieties are in terms of their morphometrical characteristics could be divided into 4 groups, where the similarities between 'Barbera', 'Pokalca', 'Refošk' and 'Syrah' varieties on the one hand, and 'Cabernet sauvignon', 'Merlot', 'Teran Istra', 'Tinta Pinheira' and 'Zweigelt' on the other being most pronounced. As far as biochemical characteristics are concerned, organic acids make only a minor contribution to the variety variability, therefore it has been recommended that in the future only individual organic acids are to be evaluated. Biochemical characteristics divide the varieties according to their similarities into 4 groups, with the most prevalent group being that with 'Barbera Bovcon', 'Cabernet sauvignon', 'Pokalca', 'Merlot', 'Zweigelt' and 'Plavina'. All evaluated morphometrical and biochemical characteristics divide the selected varieties into 4 groups, with similarities being established between 'Barbera Bovcon', 'Barbera standard' and 'Pokalca' varieties; between 'Cabernet sauvignon', 'Merlot' in 'Zweigelt' varieties; med between 'Refošk', 'Syrah' and 'Teran Istra' varieties and between 'Sladkočrn', 'Tinta Pinheira' and 'Vranac' varieties. Only 'Plovdina' fails to be classified into any one of all groups, which in turn confirms its variability in relation to other varieties.

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Agrovoc descriptors: atrazine; metabolites; coniferales; bark; herbicides; composts; leaching; degradation; soil pollution; soil profiles; groundwater pollution

Agris category code: P33, P10, T01

COBISS koda 1.01

Učinek dodanega komposta tlem na razgradnjo atrazina v kolonskem poskusu

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IZVLEČEK

V neporušenih talnih kolonah smo preučevali vpliv dodanega komposta iz lubja tlem na usodo herbicida atrazina. Ugotovili smo, da je kompost značilno vplival na hitrost razgradnje atrazina v tleh. Tri mesece po uporabi atrazina je v tleh z dodanim kompostom atrazin predstavljal 52 % vseh analiziranih substanc pesticida, medtem ko je bil njegov delež v kontrolnih tleh še 80 %. Deetilatrazin je bil glavni razgradni produkt v obeh obravnavanjih, le da je bil njegov delež v tleh z dodanim kompostom značilno večji. Nasprotno je bila zastopanost hidroksiatrazina večja v tleh brez dodatka atrazina. Razlik med obravnavanjema v prenosu atrazina skozi talni profil nismo ugotovili. Večino atrazina in metabolitov smo določili v zgornji 15 cm plasti tal, prenos v spodnje plasti t.j. do globine 45 cm je znašal manj kot 3 % skupne vsebnosti analitsko določenih ostankov herbicida v talnih in vodnih vzorcih. Izpiranje atrazina in metabolitov iz talnega profila je bilo zanemarljivo majhno (po 0,1 %), brez značilnih razlik med obravnavanjema.

Ključne besede: atrazin, metaboliti, razgradnja, izpiranje, kompost, tla, talne kolone

ABSTRACT

EFFECT OF COMPOST AMENDMENT ON THE ATRAZINE DEGRADATION IN SOIL COLUMN EXPERIMENT

Effect of conifer bark compost amendment on the fate of the herbicide atrazine in soil was examined in the undisturbed soil columns. Compost amendment affected significantly the rate of atrazine degradation in soil. Three months after atrazine application, the recovered amount of atrazine in compost amended soil represented 52 % of all analysed substances in comparison to 80 % in the control treatment. Deethylatrazine was the main metabolite in both treatments; however its share was significantly higher in the compost amended soil. In contrast, the share of hydroxyatrazine, was higher in the control soil. Differences in the atrazine transfer through the soil profile were not found. The majority of atrazine and metabolites were analysed in the upper 15 cm soil layer, mobility to the lower layers (to the depth of 45 cm) was less than 3 % of total recovered herbicide in soils and leaching waters. Leaching of atrazine and metabolites was negligible (below 0.1 %) and not significantly different between the treatments.

Key words: atrazine, metabolites, degradation, leaching, compost, soil, soil columns

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1 UVOD

Atrazin (6-kloro-4-etilamino-6-isopropilamino-1,3,5-triazin) je v svetovnem merilu eden izmed najbolj uporabljenih fitofarmaceutskih sredstev. Je selektivni herbicid, v uporabi že od leta 1958 za kontrolo širokolistnih plevelov pri pridelavi koruze, prosa, sladkornega trsa in ananasa, ter v gozdarstvu (Stevens in Sumner, 1991). Je tudi eden izmed najbolj razširjenih onesnažil podtalnic (Kolpin s sod., 1998, EEA, 1999, Tappe s sod., 2002, Guzzella s sod., 2006). Prav obremenjenost podtalnic z atrazinom in njegovimi razgradnimi produkti je vzrok za prepoved uporabe atrazina v Sloveniji. Atrazin ni več na listi registriranih fitofarmaceutskih sredstev RS od leta 2002 dalje (FURS, 2006). Vendar pa je zaradi dolgoletne in široke uporabe atrazina v RS pričakovati prisotnost te substance še več let. Rezultati monitoringov podtalnic nekaterih držav namreč kažejo, da je atrazin zelo obstojno onesnažilo, saj se lahko pojavlja v vzorcih voda tudi deset let in več po prepovedi uporabe (Guzzella s sod., 2006, Tappe s sod., 2002). Prisotnost atrazina v vodah slovenskih podtalnic v prvih letih po prepovedi t.j. od l. 2003 do l. 2005 (ARSO, 2004, 2006) to potrjujejo. Ker je atrazin v vodonosnikih povsem obstojen, je smislen razmislek o ukrepih, ki bi lahko vplivali na njegovo usodo že v tleh posebno, če je vsebnost atrazina v talnih vzorcih visoka (*hot spots*). Odločilna procesa, ki vplivata na izpiranje atrazina iz tal v podtalnico sta sorpcija (t.j. zadrževanje na talnih koloidih) ter razgradnja.

Glavna pot izginevanja atrazina v okolju je mikrobna razgradnja (Esser s sod., 1975). Poznani so številni talni mikroorganizmi, ki so sposobni delne razgradnje atrazina (Scheunert, 1992, Hickey s sod., 1994) kar vodi do nastanka in akumulacije razgradnih produktov, kot tudi mikroorganizmi, ki porabljajo hranila in energijo z mineralizacijo triazinskega obroča do CO₂ (Yanze-Kontchou in Gschwind, 1994, Struthers s sod., 1998, Topp s sod., 2000, Wackett s sod., 2002). Zelo verjetno pa je v proces razgradnje atrazina v tleh v realnih razmerah vključena kar najširša mikrobna združba s svojimi interakcijami (Smith s sod., 2005). Razpolovni čas atrazina v tleh je močno odvisen od vrste tal in lahko znaša od 2 mesecev do nekaj let (Assaf in Turco, 1994, Radosevich, 1993, Capriel s sod., 1985). V tleh sta glavni poti razgradnje atrazina *N*-dealkilacija in hidrolitična deklorinacija (Wackett s sod., 2002). Dealkilacija vodi do deetilatrizona (DEA), deizopropilatrizona (DIA) in deetildeizopropilatrizona (DDA), deklorinacija pa vodi do hidroksiatrizona (HA), hidroksideetilatrizona (DEHA), hidroksideizopropilatrizona (DIHA) in amelina. Vsi razgradni produkti so že bili analizirani v tleh, kjer je bil atrazin uporabljen, kot tudi v odcednih vodah (Scheunert, 1992).

Dodajanje organskih dodatkov tlem lahko spremeni hitrost in poti razgradnje pesticidov (Benoit in Barriuso, 1995, Hout s sod., 1998, Benoit in Preston, 2000). Organska snov na drugi strani povečuje sorpcijo pesticidov, kar znižuje njihovo biološko dostopnost (Demon s sod. 1994, Hout s sod., 1998, Gevao s sod., 2000), ter s tem tako razgradnjo kot tudi izpiranje. Namen poskusa je bil v laboratorijskem kolonskem poskusu ugotoviti vpliv izbranega organskega dodatka tlem t.j. komposta iz lubja na razgradnjo in izpiranje atrazina.

2 MATERIAL

2.1 Tla

Izbrali smo obrečna tla na travniku v Zg. Konjišču v Apaški dolini, kjer še ni bil uporabljen atrazin. Neporušene talne vzorce smo odvzeli z zaporednim zabijanjem 10 cm kolotov v talnem profilu (A, B, C in D plast). Lastnosti tal v posameznih plasteh so predstavljene v tabeli 1.

Preglednica 1: Pomembnejše lastnosti tal pred dodajanjem komposta

Table 1: Selected properties of soils before adding compost

Globina tal v koloni	pH	org. sn.	pesek	melj	glina	Teksturni razred
Soil depth in the column	pH	org.matt.	sand	silt	clay	Texture Class
	(KCl)	%	%	%	%	
A. 0-10 cm	7.2	3.2	62	31	7	PI / SaL
B. 10-20 cm	7.6	2.0	59	33	8	PI / SaL
C. 20-30 cm	7.8	1.7	50	42	8	I / L
D. 30-40 cm	7.8	1.4	37	54	9	MI / SiL

2.2 Kompost

V laboratoriju smo v zgornjo 10 cm plast tal zamešali kompost iz lubja z naslednjimi lastnostmi: 77 % organske snovi, 0,65 % dušika, 12,5 mg K/100 g, 7,9 mg P₂O₅/100g, pH (KCl) 6,9, CEC 190 mmol/100g. Količino dodanega komposta smo določili na osnovi kationske izmenjalne kapacitete (CEC), ki smo jo v zgornjem 10 cm sloju z dodatkom komposta podvojili iz 25 na 55 mmol/100g tal. Vsebnost organske snovi se je povečala iz 3,2 na 8,0 %. Kompost je bil dodan v odmerku 325 g suhega komposta na kolono oz. 18 kg/m².

2.3. Atrazin

Na površino talne kolone smo kapljično nanesti 10 ml raztopine pripravka Gesaprim 50 WP, ki vsebuje 50 % aktivne snovi (a.s.) atrazina. Odmerek atrazina na kolono je bil 5,4 mg a.s., kar je skladno s poljskim odmerkom 3 kg a.s./ha.

3 METODE

3.1 Kolonski poskus

Kolone s premerom 15,2 cm in dolžine 60 cm smo sestavili v laboratoriju iz odsekov juvidurne cevi napolnjenih z neporušenim vzorcem tal. Le te smo odvzeli zaporedoma z enakomernim zabijanjem 10 cm odsekov juvidurne cevi v talni profil na lokaciji Zgornje Konjišče v Apaški dolini. Spodnji 10 cm odsek kolone smo napolnili s kremenčevim peskom. Med posameznimi odseki smo namestili juvidurne obroče in odseke povezali s silikonom. Zgornji, površinski del talnega profila smo prekrili s stekleno volno. Ker se je ob dodajanju komposta v zgornjo 10 cm plast tal višina te plasti povečala na 15 cm, smo v kontrolnih kolonah zgornjo plast tal povečali do enake višine s homogeniziranim vzorcem tal zgornje 10 cm plasti talnega profila. Pred začetkom poskusa smo kolone navlažili do poljske kapacitete s kapilarnim dvigom vode in nato mesec dni simulirali padavine z namenom stabilizacije kolon. Zalivali smo trikrat tedensko po 130 ml/kolono, kar je ekvivalentno 96 mm/mesec. Sistem zalivanja kolon smo izbrali na osnovi 25 letnega povprečja padavin v mesecih maj, junij in julij v Apaški dolini. Po nanosu atrazina na površino tal je poskus tekel 104 dni. Odcedne vode smo zbirali v 400 ml odmerkih in jih sproti analizirali na vsebnost atrazina in metabolitov. Po končanem poskusu smo kolone razdri po plasteh (glej točko 2.1), jih zračno posušili in homogenizirali. Do analiziranja smo vzorce tal zamrzili. Vsako obravnavanje smo izvedli v treh ponovitvah.

3.2 Določanje atrazina in razgradnih produktov

Zatehtali smo 10 g homogeniziranih tal, jih suspendirali v 20 ml topila (metanol / voda = 9 / 1) in dali v ultrazvočno kopel za 10 min (25-30 °C). Ekstrakt smo odločili s centrifugiranjem in nato uparili na rotavaporju. Ekstrakcijo in uparjevanje smo ponovili še dvakrat. Posušen vzorec smo raztopili v 1 ml acetonitril acetatnega pufra.

Vzorci odcednih voda (400 ml) smo ekstrahirali v 3 ml kolonah z 200 mg trdne faze (LiChrolut EN, Merck). Ekstrakte smo eluirali z metanolom. Topilo smo odparili na rotavaporju in posušen vzorec raztopili v 200 μ l acetonitril-acetatnega pufru. V vseh ekstraktih smo določili atrazin in razgradne produkte: DEA, DIA, DDA, DEHA, HA in DIHA s HPLC (Perkin Elmer 235) pod sledečimi pogoji: detektor: Diode Array Perkin Elmer, detekcija pri 220 in 240 nm; predkolona: LiChrocart 4 – 4, Lichrospher RP-select B, kolona: LiChrocart 250 – 4, Lichrospher RP-select B; pretok: 1,5 ml/min; mobilna faza: A = acetonitril, B = acetatni pufer (0,002 M, pH=7,0-7,3); gradient za talne vzorce: 5,5 min 3 % A izokratsko, 5 min 35 % A linearno, 7,5 min 35 % A izokratsko, 7 min 3 % A stopničasto; gradient za vodne vzorce: 3 min 24 %, 1 min 24-35 % A, 9 min 35 % A, 1 min 35-24 % A, 6 min 24 % A.

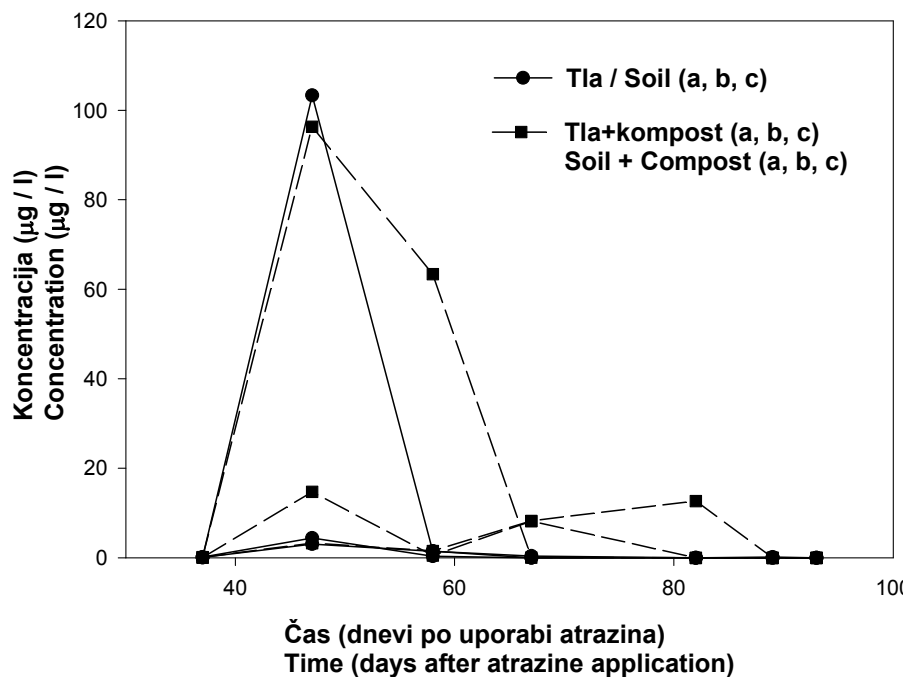
4 REZULTATI Z RAZPRAVO

Tri mesece po uporabi atrazina smo v obeh obravnavanjih večino atrazina in metabolitov določili v zgornji 15 cm plasti tal (Tabela 2). Prenos herbicida v spodnje plasti tal (B, C in D) je znašal manj kot 3 % skupne vsebnosti analitsko določenih ostankov herbicida v talnih in vodnih vzorcih (atrazina in metabolitov). Izpiranje atrazina in metabolitov iz talnega profila je bilo v obdobju treh mesecev po uporabi atrazina zanemarljivo majhno (<0,1 % analitsko določenih ostankov herbicida), kar je skladno z ugotovitvami drugih avtorjev, ki so preučevali izpiranje triazinskih herbicidov (Kruger, 1993; Scheunert s sod., 1994; Langenbach s sod., 2000). Herbicid se je pojavil v odcednih vodah 37. dan po uporabi atrazina, največje koncentracije pa smo zaznali 47. dan (Slika 1). V obeh obravnavanjih smo v eni izmed treh ponovitev zabeležili dogodek z koncentracijo atrazina v odcednih vodah okoli 100 μ g/l. Vsebnost metabolitov v odcednih vodah je bila ves čas poskusa pod detekcijsko mejo.

Preglednica 2: Razporeditev atrazina in metabolitov po pozameznih plasteh talne kolone in v odcednih vodah tri mesece po nanosu aktivne snovi (% skupne vsebnosti analitsko določenih ostankov herbicida v talnih in vodnih vzorcih). Predstavljeno je povprečje in standardni odklon treh ponovitev.

Table 2: The % of the recovered atrazine and its metabolites in the soil layers and leachates three months after active ingredient application. Averages and standard deviation of three replicates are presented.

Obravnavanje Treatment		Atrazin in metaboliti (%) Atrazine and metabolites (%)	
		Tla Soil	Tla + kompost Soil + Compost
T S	A plast / layer	98,8 ±0,5	97,0 ±0,9
L O	B plast / layer	0,9 ±0,6	2,8 ±0,9
A I	C plast / layer	0,3 ±0,1	0,3 ±0,1
L	D plast / layer	0 ±0	0 ±0
Odcedne vode Leaching water		0,003 ±0,005	0,006 ±0,007

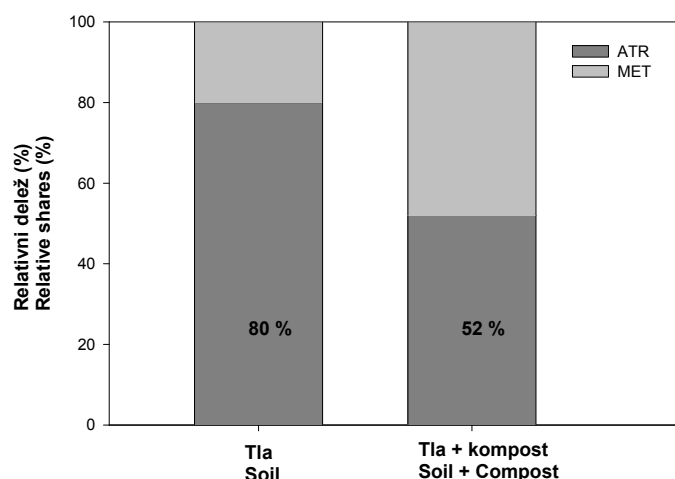


Slika 1: Skupna vsebnost atrazina in metabolitov ($\mu\text{g/l}$) v odcednih vodah kolonskega poskusa v odvisnosti od časa po uporabi atrazina. Prikazane so tri ponovitve (a, b, c) obeh obravnavanj (tla in tla z dodatkom komposta).

Figure 1: Total atrazine and metabolites content ($\mu\text{g/l}$) in the leaching waters of column experiment in dependence of time following atrazine application. Three replicates (a, b, c) of both treatments (soil and soil with compost amendment) are presented.

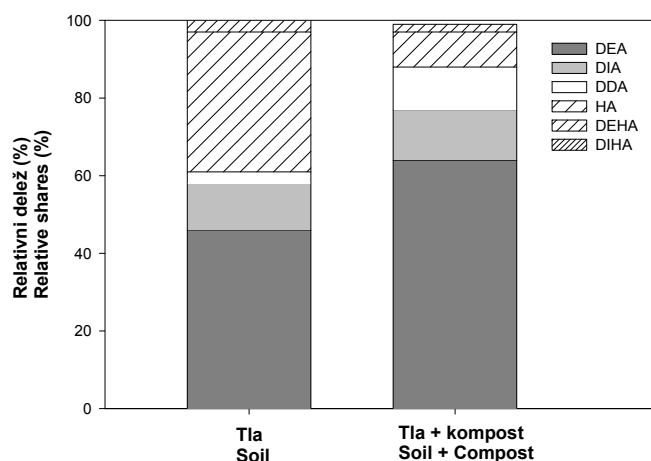
Največje razlike med obravnavanjema smo ugotovili v razmerju med vsebnostjo analitsko določenega atrazina in skupno vsebnostjo analitsko določenih metabolitov (Slika 2). Ugotovili smo, da dodajanje komposta tlem povečuje razgradnjo atrazina. Tri mesece po uporabi atrazina smo v kontrolnih tleh določili 80 % atrazina, medtem ko v tleh z dodatkom komposta le 52 % od skupne vsebnosti atrazina in metabolitov (Slika 2). Razlika med obravnavanjema je bila statistično značilna (Tukeyev test, $p \leq 0,05$).

Dodani kompost je nadalje vplival na strukturo razgradnih produktov atrazina v tleh (Slika 3). V obeh obravnavanjih je sicer največji delež analiziranih metabolitov predstavljal deetilatrazin (DEA), le da je njegov delež v tleh z dodanim kompostom statistično značilno večji (Tukeyev test, $p \leq 0,05$). Domnevamo, da je bila glavna pot razgradnje v obeh obravnavanjih *N*-dealkilacija, ker so deetilatrazin (DEA), deizopropilatrazina (DIA) in deetildeizopropilatrazina (DDA) predstavljali več kot 50 % analiziranih metabolitov. Velik delež hidroksi atrazina (HA) v kontrolnih tleh, kot tudi značilne razlike v deležih deetilatrazina (DEA), nakazujeta na razlike med obravnavanjema v poteh in ne le v hitrosti razgradnje atrazina. Obe metabolni poti, *N*-dealkilacija in hidrolitična deklorinacija, pa sta lahko mikrobiološko pogojeni (Wackett s sod., 2002).



Slika 2: Vsebnost atrazina (ATR) in skupna vsebnost metabolitov (MET): DEA, DIA, DDA, DEHA, HA in DIHA analitsko določenih v talnih vzorcih kolonskega poskusa tri mesece po uporabi atrazina. Predstavljeno je povprečje in standardni odklon treh ponovitev.

Figure 2: Atrazine (ATR) and sum of metabolites contents (MET): DEA, DIA, DDA, DEHA, HA and DIHA determined in the soil samples of column experiment three months after atrazine application. Average and standard deviation of three replicates is presented.



Slika 3: Razmerje metabolitov: DEA, DIA, DDA, DEHA, HA in DIHA analitsko določenih v talnih vzorcih kolonskega poskusa tri mesece po uporabi atrazina.

Figure 3: Ratios between atrazine metabolites: DEA, DIA, DDA, DEHA, HA in DIHA determined in the soil samples of column experiment three months after atrazine application.

Potencial dodanega komposta za povečevanje razgradnje atrazina iz tal sta dokazala že Benoit in Preston (2000), ki sta ugotovila značilno povečanje mineralizacije atrazina v tleh z dodatkom kompostirane strniščne slame (50% v primerjavi s kontrolnimi 15 %). Abdelhafid s sodelavci (2000) je ugotovil, da dodatek kompostiranega blata komunalne čistilne naprave povečuje razgradnjo atrazina v tleh, vendar le v obravnavanju, kjer ni bilo prisotne atrazinu prilagojene mikrobne združbe. Ker smo v poskusu uporabili tla, kjer atrazin v preteklosti ni bil uporabljen, sklepamo, da tudi v naših tleh atrazinu prilagojene mikrobne združbe ob začetku poskusa ni bilo. Houot s sodelavci (1998) pa vpliva dodanega komposta (kompostiranega blata komunalne čistilne naprave in kompostirane strniščne slame) na razgradnjo atrazina ni ugotovil, medtem ko je dodatek obeh vrst kompostov značilno povečal sorpcijo atrazina v tleh.

Potrebno je poudariti, da se kompostni materiali različnih študij medsebojno razlikujejo po izvoru, sestavi in odmerkih, zato rezultati niso direktno primerljivi. Poleg tega je vpliv komposta na razgradnjo pesticidov v tleh zelo kompleksen. Toliko bolj je potrebno za ločevanje procesov sorpcije (predvsem vezanih ostankov pesticida) in popolne razgradnje (mineralizacije) izvajati poskuse s ¹⁴C-označenimi pesticidi, kar je pomanjkljivost naše študije. V splošnem se z vnosom organske snovi povečuje sposobnost tal za sorpcijo pesticidov, ki je odvisna od vrste in zrelosti le te (Benoit s sod., 1996, Benoit in Preston, 2000, Gevao s sod., 2000, Spark in Swift, 2002). Na ta način se predvsem z nastankom vezanih ostankov (*bound residues*) lahko začasno zmanjša dostopnost pesticida za razgradnjo. Po drugi strani pa dodatek razgradljivih oblik C lahko stimulira ko-metabolično biološko razgradnjo pesticidov preko povečane mikrobiološke aktivnosti (Hance, 1973, Topp s sod., 1996, Wanner s sod., 2005). Domnevamo, da je dodatek izbranega komposta iz lubja vplival na pestrost in aktivnost mikrobne združbe, kar se je pokazalo v manjši vsebnosti atrazina v tleh v primerjavi s kontrolnim obravnavanjem (Slika 2).

Zaradi preobremenjenosti predvsem vodnih vzorcev v Sloveniji z atrazinom, so pomembne tudi ugotovitve avtorjev Tsui in Roy-a (2006), ki ugotavljata, da kompost zelenih odpadkov odstranjuje atrazin iz raztopin, pravtako zaradi stimulativnega vpliva na procesa sorpcije in razgradnje.

6 SKLEPI

Dodatek komposta iz lubja tlem je pospešil razgradnjo atrazina in vplival na pot njegove razgradnje. V splošnem bi kompost lahko bil potencialni filterski material za odstranjevanje atrazina iz tal, kot tudi iz raztopin, vendar manjkajo sistematične študije vpliva vrste komposta na aktivnost mikrobnih združb, biološko razgradnjo ter sorpcijo atrazina.

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Agrovoc descriptors: pasta; processed plant products; cereal products; plantago; guar gum; buckwheat; noncereal flours; fagopyrum tataricum; fagopyrum esculentum; flavour; organoleptic properties; quality

Agris category code: Q02, Q04

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Izdelava testenin iz mok navadne in tatarske ajde

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IZVLEČEK

Predstavljeni so rezultati poskusov izdelave testenin iz mok navadne in tatarske ajde. Dodatek psylliuma testenin ne izboljša, testenine z dodatkom moke guara (5%) pa so v primerjani s testeninami brez tega dodatka manj občutljive na mehanične poškodbe. Testenine iz tatarske ajde so zelenkasto rumeno rjave barve, medtem ko so testenine iz navadne ajde svetle rumenkasto rjave barve.

Ključne besede: testenine, psyllium, guar, navadna ajda, tartarska ajda

ABSTRACT

PRODUCTION OF PASTA FROM COMMON AND TARTARY BUCKWHEAT FLOURS

Efforts to produce pasta from common and tartary buckwheat flour samples are presented. Pasta with addition of psyllium were not better in comparison to pasta without this addition. However pasta with the guar flour addition (5%) was more resistant to breakage. Pasta from tartary buckwheat flour was greenish yellow-brown in comparison to light yellowish brown pasta from common buckwheat flour.

Key words: pasta, psyllium, guar, common buckwheat, tartary buckwheat

1 UVOD

Izdelava testenin iz čiste ajdove moke ni pogosta tehnologija pri pripravi testenin. Večinoma se proizvajalci odločajo za različne kombinacije mešanic med ajdovo in pšenično moko, v katerih pšenična moka prevladuje. Testenine iz pšenične moke z dodatkom ajdove moke so dodatna ponudba proizvajalcev, ne pa tudi dobro prodajan izdelek. Tehnologija proizvodnje testenin iz čiste ajdove moke je kompleksno zahtevnejša, saj se ajdova moka v tehnologiji zaradi svojih karakteristik značilno drugače obnaša. Zaradi tega se taka izdelava testenin redko uporablja. Zanimiva pa postaja predvsem v segmentu prehrane, kjer se zahteva odsotnost glutena. Vedno bolj

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priljubljeni postajajo izdelki iz ajdove moke tudi zaradi vsebnosti v zadnjih letih odkritih zdravju prijaznih sestavin .

Namen raziskave je bil eksperimentalno preučiti tehnologijo izdelave testenin iz čiste ajdove moke ter iz ajdove moke z dodatkom guarjeve moke ali dodatkom moke psylliuma. Želeli smo ovrednotiti tehnološki postopek izdelave testenin ter preučiti nekatere tehnološke parametre. Namen je bil spremljati obnašanje vzorcev ajdovega testa pri mesenju ter izdelavi standardnih svežih testenin (špageti). Nadalje je bil namen preučevati obnašanje vzorcev testenin med sušenjem v eksperimentalni sušilnici ter senzorične lastnosti posušenih testenin. Nadalje smo primerjali značilnosti in obnašanje test in končnih izdelkov (testenin) pri uporabi različnih vrst ajdovih mok ter z dodatki guarjeve moke in psylliuma.

Cilj raziskave je bil spoznati razlike v tehnoloških postopkih izdelave testenin glede na vrsto ajdove moke ter spremembe v obnašanju test in končnih izdelkov ob dodajanju guarjeve moke ali psylliuma v praškasti obliki.

2 PREGLED VIROV

Guar, *Cyamopsis tetragonoloba* (L.) Taub. je leguminoza. Semena so sestavljena iz luske, s polisaharidi, bogatega endosperma in kalčka, ki ima visoko vsebnost beljakovin (www.texturant-systems.com/texturant/html/e/products/guar.htm). Rastlina najpogosteje raste v Pakistanu in severni Indiji. Tam se mladi stroki uporabljajo tudi za prehrano ljudi in živali. Seme (zrno) guarja je bogato z galaktomanani (Stevens, 2003), dolgoverižnimi polisaharidi sestavljenimi iz večjega števila galaktoz in manoz (<http://scifoys.com/ingredients/guargum.html>).

Guarjeva moka je lahko topna v vodi, ima relativno visoko viskoznost v majhnih koncentracijah ter pH med 5,5 – 6,1 (Fennomena, 1996). Iz literature je znano, da se guarjeva moka uporablja tudi komercialno v živilski proizvodnji, kot polnilo oz. gostilo ali emulgator. Zamenja lahko koruzni škrob, moko tapioke, itd. (www.wisegeek.com). Ima bistveno večjo moč zgoščanja kot koruzni škrob, zato je popularen dodatek predvsem v pudingih in sladolednih kremah. Guarjevo moko bi lahko opisali kot naravno gostilo, ki se uporablja v živilskih procesih pri proizvodnji hrane. Uporablja se tudi v dresingih, omakah, mlečnih izdelkih, pekarstvu, pa tudi v farmacevtski in kozmetični industriji, v papirni in tekstilni industriji (www.foodreference.com).

Guarjeva moka ni samo gostilo, ampak tudi sredstvo za vezanje in plastifikator. Uporablja se v majhnih količinah kot dodatek, prevelike količine užitega guarja pa bi lahko povzročile tudi motnje v delovanju intestinalnega trakta (www.wisegeek.com). Guarjeva moka je deklarirana kot EU aditiv E 412. Popraševanje po guarjevi moki narašča, zato se tudi območja gojenja te rastline širijo.

Genus *Plantago* ima več kot 200 vrst (speciesov). Ovata trpotec (*Plantago ovata*) je rastlina, ki raste samoniklo v Iranu in Indiji, tam in v sosednjih državah jo tudi gojijo. Enako učinkovit je indijski trpotec (*P. indica*), ki je doma tudi pri nas na peščenih tleh v okolici Celja, gojijo pa ga predvsem v Franciji. Ovata trpotec (*P. ovata*) ter vrsto trpotca psyllium (*P. psyllium*) pridelujejo komercialno v več evropskih državah in državah bivše SZ, pa tudi v Pakistanu in Indiji. Na Biotehniški fakulteti Univerze v

Ljubljani so ga poskušali pridelovati na laboratorijskem polju ter pri kooperantu na Dolenjskem (Vrh nad Višnjo goro), a je dal le skromen pridelek (osebna informacija, I. Kreft).

Indijski trpotec spada v kategorijo H, ki ima enak pravni položaj v naši zakonodaji kot hrana (Špringer, 2003).

V zadnjih časih zanimanje za psyllium narašča predvsem zaradi vsebnosti vlaknin ter nekaterih zdravju prijaznih lastnosti, ki mu jih pripisujejo zaradi vsebnosti vlaknin (npr. zniževanje holesterola, zniževanje glukoze v krvi). Seme trpotcev se komercialno uporablja za proizvodnjo pripravka, ki ima lastnosti tvorjenja sluzi z želatinoznimi značilnostmi in lahko deluje kot odvajalo. Lupina psylliuma je neenergijski vir vlaknin, ki podpira zdravo prebavo, ima vpliv na krvni tlak in nivo holesterola v krvi. Že nekaj časa se uporablja v ayurveda medicini. Raziskave tudi nakazujejo, da je psyllium, vključen kot dodatek v živila, bolj učinkovit v svojih zdravju prijaznih lastnostih kot ločen od hrane. Poznamo pa tudi primere alergičnih reakcij pri uživanju žit, ki vsebujejo dodatke psylliuma.

Lupina psylliuma se sestoji iz hemiceluloze, ki deluje v prebavilih kot goba, izboljšuje konsistenco črevesne vsebine, pospešuje peristaltiko črevesja in potovanje hrane skozi intestinalni trakt. Njeno delovanje je čisto mehanično – človeško telo ga ne absorbira in se zato lahko uporablja kot laksativ brez tveganja za odvisnost ali toksičnosti. Priporoča se do 5 g psylliuma na dan. (www.supersmart.com) oziroma po priporočilih farmacevtov 5-15g indijskega trpotca dnevno, v primerih akutnih drisk pa tudi do 40 g (Špringer, 2003).

Sluzi ne delujejo sistemsko, ker se ne prebavijo in vsrkajo, le deloma jih razcepijo bakterije v debelem črevesu. Ne dražijo črevesne sluznice in ne povzročijo navajenosti po daljši rabi. Preobčutljivost za drogo je redka, strupenost pa zanemarljiva (Špringer, 2003).

Psyllium pridelujejo predvsem zaradi vsebnosti vlaknin, ki se značilno sluzasto obnašajo in so temeljna sestavina, ki jih pridobivamo iz rastline. Te sestavine se nahajajo večinoma v lupini. Pridobivamo jih z mehanskim mletjem semen ter ločevanjem delcev lupine od ostalih delcev zrna. Lupina predstavlja približno 25% ali celo več celotne mase zrna. Zmlete luske so bela substanca z veliko vsebnostjo vlaknin ter imajo hidrofilne lastnosti (vežejo vodo). Po absorpciji vode volumen prozorne brezbarvne želatinozne sluzi naraste celo do 10 krat in več. Psyllium se v glavnem uporablja kot dietna vlaknina, ki se ne prebavlja v tankem črevesju.

ZDA so glavni uvoznik psylliuma (predvsem lupine) za farmacevtsko industrijo za proizvode kot npr. »Metamucil«, "Effersyllium" and "Fiberall". Psylliumove vlaknine se uporabljajo tudi kot naravna dietna vlaknina za prehrano živali. Seme brez lupine, ki ostane po mletju zunanje plasti zrna, je bogato s škrobom in maščobnimi kislinami in se tudi uporablja za živalsko krmo.

Psylliumove želatinozne substance imajo tudi druge zelene lastnosti. Delujejo lahko tudi kot gostilo v sladolelih in zamrznjenih desertih, pa tudi pri drugih živilih. Že 1,5% psylliuma ima približno takšne vezne lastnosti v živilih kot 10% škroba. Psyllium lahko ima tudi viskozne lastnosti, ki pa so povezane in odvisne od temperature, pH vrednosti medija in koncentracije soli. Te lastnosti v kombinaciji s

karakteristikami naravnih vlaknin lahko pomenijo postopno večje zanimanje za psyllium v živilski industriji in živilsko tehnoloških procesih (Hansen in sod., 1992).

3 MATERIAL IN METODE DE LA

Material

Kot material smo uporabili 3 vzorce ajdovih mok in sicer:

- klasično mleto ajdovo moko iz navadne ajde iz mlina Korošec (12,1% beljakovin / SS) (vzorec 1),
- ajdovo moko iz navadne ajde, francoskega porekla, pridelana v okolici Šentjerneja na Dolenjskem , zmleta na mlin na mlinske kamne (proizvajalec Rangus) (14,8% beljakovin / SS) (vzorec 2),
- tatarsko ajdovo moko iz Luksemburga (7,9 % beljakovin / SS) (vzorec 3).

Metode dela

Tehnološki postopek izdelave testenin je potekal v eksperimentalnem laboratoriju Živilske šole Maribor. Recepture in navodila za izdelavo testenin so lastno delo in so bili predhodno preizkušeni pri izdelavi testenin iz pšenične moke ter ajdove moke dostopne za nakup v trgovski mreži.

Uporabljena je bila laboratorijska eksperimentalna oprema za proizvodnjo testenin (stroj za testenine Gostol, tip Dolly ter univerzalni eksperimentalni sušilnik Kambič, tip SP – 190 C z lastno prilagoditvijo za sušenje testenin). Za senzorična ocenjevanja in degustacije smo uporabili prilagojene načine ocenjevanja.

4 NAČRT POSKUSA

Poskus je potekal po naslednjem načrtu:

- a) izbira in priprava receptur za izdelavo testenin (špageti),
- b) izdelava špagetov iz 3 vzorcev ajdovih mok (vzorci 1, 2 in 3),
- c) izdelava špagetov iz 3 vzorcev ajdovih mok z dodatkom 5% guarjeve moke (vzorci 1G, 2G in 3G),
- d) izdelava špagetov iz 3 vzorcev ajdovih mok z dodatkom 2 % moke psylliuma - preparat Mucilar (vzorci 1P, 2P in 3P).

5 REZULTATI

Postopek izdelave testenin brez dodatkov (primerjaj razpredelnico 1):

V kotličku mešamo 500 g moke in 5 jajc tako dolgo, da se moka enakomerno poveže z jajci v testo. Čas mešanja je 10 minut. Ugotavljamo glede na izkušnje in predposkuse, da je čas mešanja nekoliko daljši kot bi bil, če bi uporabili idealno količino moke za uporabljeni mešalnik (1 kg), vendar smo bili omejeni s količino vzorca. Mešalnik ima klasično obliko lopatic za testo. Ob potencialni uporabi drugačne oblike lopatic bi lahko nekoliko skrajšali čas mešanja. Testo po mešanju mora biti gladko, sipko in ne sme biti v kepah, kar je pomembno za izdelovanje testenin na potisk. Temperatura testa je odvisna od časa mešanja, pa tudi od števila mešanj oziroma večkratne zaporedne uporabe stroja, saj se segreva tudi kotliček za testo in model, skozi katerega polž potiska testo. Temperatura eksperimentalnih test v naših poskusih se je gibala od 21 do 23 °C. Špageti, ki jih potiska polž skozi model

št. 7 so zelo rahli in gladki. Obešajo se na palčke in sušijo v sušilniku 8 ur pri temperaturi 30 °C.

Razpredelnica 1. Osnovna planirana receptura:

Vrsta ajdove moke	Vzorec 1	Vzorec 2	Vzorec 3
Masa moke (g)	500	500	500
Masa jajc (g)	257	261	258
Jajca (kom)*	5	5	5
Čas mešanja (min)	10	10	10
Temperatura testa (°C)	23	21	21
Čas sušenja (h)	8	8	8
Temperatura sušenja (°C)	30	30	30
Ventilacija (%)	30	30	30
% vode	9,14	9,93	9,05

*1 jajce z lupino je približno 59 g, 1 jajce brez lupine (rumenjaki in beljaki) je približno 51-52 g.

5.1 REZULTATI IZDELAVE TESTENIN IZ TREH VZORCEV AJDOVIH MOK

Vzorec 1:

Sveži špageti (velikost šob - model št. 7) so zelo rahli in gladki. So rumeno rjave barve. Ob zelo previdnem obešanju špagetov na palčke so se špageti po 20 minutah že pred sušenjem v sušilniku začeli trgati. Po 55 minutah se je večina špagetov potrgala in so padli na mrežo. Tako smo jih sušili naprej. Po sušenju je zelo malo špagetov ostalo na palčki. Na dotik so bili zelo lomljivi.

Vzorec 2 :

Pri istem razmerju med moko in jajci je zamešano testo bolj kepasto od ostalih dveh (vzorec 1 in 3). Sveži špageti so bolj elastični. Lahko jih obesimo na palčke. Po 125 minutah v sušilniku so se začeli špageti lomiti. Na upognjenem mestu pa niso vsi špageti nalomljeni. Po končanem sušenju je pri vzorcu 2 (v primerjavi med vsemi 3 vzorci) največ špagetov ostalo na palčkih. Tudi ti špageti so na dotik zelo lomljivi.

Vzorec 3:

Izdelani sveži špageti so zeleno rumeno rjave barve. Špageti so bolj gladki kot pri vzorcu 1 zaradi fine granulacije moke (bolj fina granulacija moke kot v vzorcu 1). Pretrgajo se takoj, ko jih obesimo na palčko. Tako špagetov sploh ni možno sušiti na palčkih. Špagete v celoti posušimo na mreži in na dotik so zelo lomljivi.

5.2 REZULTATI IZDELAVE TESTENIN IZ VZORCEV AJDOVIH MOK Z DODATKOM GUARJEVE MOKE

Postopek izdelave testenin z dodatkom guarjeve moke (primerjaj razpredelnico 2):

Ajdovo moko in guarjevo moko smo skupaj presejali ter temeljito premešali. Moka in jajca so se mešali tako dolgo, dokler se moka ni enakomerno povezala z jajci v gladko testo. V sipkosti in gladkosti testa med vzorci ajdove moke ni bistvenih razlik. Temperatura eksperimentalnih test v teh poskusih se je gibala od 26 do 29,3 °C. Špageti, ki jih potiska polž skozi model št. 7 so zelo rahli in gladki. Obešajo se na palčke in sušijo v sušilniku 6 ur pri temperaturi 40 °C.

Razpredelnica 2. Osnovna planirana receptura pri dodatku guarjeve moke:

Vrsta ajdove moke	Vzorec 1G	Vzorec 2G	Vzorec 3G
Masa moke (g)	500	1000	500
Masa jajc (g)	299	555	285
Jajca (kom)	5,5	10,5	5,5
Čas mešanja (min)	10	10	10
Temperatura testa (°C)	29,3	26	28,3
Čas sušenja (h)	6	6	6
Temperatura sušenja (°C)	40	40	40
Ventilacija (%)	30	30	30
% vode	ni podatka		
Guarjeva moka v g (5%)	25	50	25

Vzorec 1G:

Izdelani sveži špageti so rumeno rjave barve, gladki ter elastični na dotik. Pri obešanju na palčke se niso trgali. Tudi na palčkah se ni videla natrganost špagetov. Po sušenju so bili špageti trdni in elastični. Vsi špageti so ostali na palčkah. Tudi na dotik niso bili tako krhki kot vzorci brez dodatka guarjeve moke in smo jih lahko pakirali.

Vzorec 2G:

Izdelani sveži špageti so rjave barve in na izgled so stekleni. Pri obešanju špagetov na palčke se čuti elastičnost in špageti se ne trgajo. Po 90 minutah na palčkah špageti še niso niti malo natrgani. Po sušenju so vsi špageti ostali na palčkah obešeni. Na dotik so posušeni špageti trdni in elastični.

Vzorec 3G:

Pri pripravi testa ni nobenih bistvenih razlik.

Izdelani špageti so zeleno, rumeno, rjave barve. Pri obešanju svežih špagetov na palčke so se nekateri pretrgali. 45 minut po izdelavi špagetov pred sušenjem je več kot polovica natrganih. Po sušenju je stanje nespremenjeno glede napokanosti testenin. Posušeni špageti so trdni in elastični.

5.3 REZULTATI IZDELAVE TESTENIN IZ VZORCEV AJDOVIH MOK Z DODATKOM PSYLLIUMA (preparat MUCILAR)

Postopek izdelave testenin z dodatkom Mucilarja (primerjaj razpredelnico 3):

Ajdovo moko in psyllium moko (preparat Mucilar) smo skupaj presejali ter temeljito premešali. Moka in jajca so se mešali tako dolgo, dokler se moka ni enakomerno povezala z jajci v gladko testo. V sipkosti in gladkosti testa tudi med temi vzorci ajdove moke ni bistvenih razlik. Temperatura eksperimentalnih test v naših poskusih je bila od 21,0 do 23,7°C. Špageti, ki jih potiska polž skozi model št. 7 so zelo rahli in gladki. Obešajo se na palčke in sušijo v sušilniku 6 ur pri temperaturi 40 °C.

Razpredelnica 3. Osnovna planirana receptura pri dodatku moke psylliuma:

Vrsta ajdove moke	Vzorec 1P	Vzorec 2P	Vzorec 3P
Masa moke (g)	540	531,8	500
Masa jajc (g)	266,35	256	266
Jajca (kom)*			
Čas mešanja (min)	15	15	15
Temperatura testa (°C)	23,7	21,0	22,5
Čas sušenja (h)	6	6	6
Temperatura sušenja (°C)	40	40	40
Ventilacija (%)	30	30	30
% vode	ni podatka		
Psyllium v g (2%)	10,01	10,25	10

*1 jajce je približno 51 – 52 g (brez lupine)

Vzorec 1P:

Izdelani sveži špageti so rahli. Po obešanju špagetov na palčke so se kmalu natrgali. Eno uro po izdelavi je več kot polovica špagetov, ki so bili obešeni, pretrganih. Po sušenju se špageti niso dodatno lomili. Na dotik pa so špageti rahli.

Vzorec 2P:

Izdelani sveži špageti so rahli in nekoliko bolj elastični od vzorcev 1 in 3. Špageti po izdelavi in pred sušenjem se delno natrgajo, ko jih obesimo na palčke. Po sušenju je nekaj testenin polomljenih in so na dotik izjemno rahle.

Vzorec 3P:

Izdelani sveži špageti so gladki in rahli. Špagete smo lahko obesili na palčke, vendar so se kmalu začeli trgati. Špagetov nismo mogli posušiti na palčkah ampak na mreži. Posušeni špageti so na dotik rahli in lomljivi.

6 DISKUSIJA

Pri izdelavi testa smo najprej ugotavljali sposobnost mešanja osnovnih surovin, to je jajc in ajdove moke. Ugotavljali smo sposobnost zamesitve v testo ter kakovost

zamesitve. Preučevali smo enakomernost gostote testa, sprijemanje v kepe ali grude oziroma enakomernost in gladkost zamesi.

Preučili in primerjali smo obnašanje 3 različnih vrst ajdovih mok za izdelavo testenin na potisk. Ugotovili smo, da je najprimernejše razmerje med maso katerekoli ajdove moke in maso jajc 2 : 1 (na 500 g ajdove moke je 5 – 5,5 jajc oz. na 100 g ajdove moke 1 jajce (približno 51-52,5 g beljaka in rumenjaka). Struktura testa je bila najbolj kepasta pri vzorcu 2, če primerjamo vse tri vzorce med seboj.

Barva svežih testenin (špagetov) se razlikuje med vzorci. Pri vzorcih navadne ajde (vzorec 1 in 2) so sveže testenine rumeno rjave, pri vzorcu testenin iz tatarske ajde pa zeleno rumeno rjave, kar loči tatarske testenine barvno od ostalih dveh vzorcev.

Sveži špageti so bili pri vseh vzorcih izjemno rahli in gladki. Pri obešanju na palčke za sušenje, tega tretmana vzorci iz tatarske ajde (vzorec 3) sploh niso prenesli, saj so se večinoma takoj pretrgali, tako da smo te vzorce posušili na mreži. Pri poskusu sušenja svežih špagetov iz vzorcev navadne ajdove moke (vzorec 1 in 2) ugotavljamo, da so se vzorci 1 pričeli trgati po približno 20 minutah, nadalje v eni uri pa se je pretrgala večina vzorcev. Le redki so ostali na palčkah za sušenje po končanem sušenju. Medtem ko so se špageti iz vzorca 2 obnašali najboljše. Bili so najbolj elastični. Po 45 minutah sušenja so se pričeli prav tako lomiti, kljub temu pa je na palčkah ostalo nekaj več špagetov kot pri vzorcu 1.

Ugotavljamo pa, da so bili posušeni špageti izjemno krhki in lomljivi ter neprimerni za pakiranje in transport.

Ob dodatku guarjeve moke nismo med testi ugotavljali nobenih razlik pri pripravi testa. Pojavljajo pa se bistvene razlike pri svežih testeninah (špagetih), saj nastaja viden napredek v elastičnosti svežih špagetov, ki jih v vseh treh primerih lažje obešamo na palčke za sušenje in pri vseh treh vzorcih se vidi nek napredek v elastičnosti v primerjavi z istim vzorcem brez dodatka guarjeve moke. Tako na primer vzorec 3G (špageti iz tatarske ajde z dodatkom guarjeve moke) uspemo obesiti na palčke za sušenje, čeprav se po določenem času pričnejo trgati. Vzorec 1G in 2G pa uspemo posušiti na palčkah. Ugotavljamo, da z dodatkom guarjeve moke sveži špageti pridobijo na elastičnosti, nekoliko pa se zmanjša tudi trganje špagetov med sušenjem.

Spremeni se tudi struktura posušениh špagetov, saj ti na dotik niso več zelo občutljivi in lomljivi, ampak postanejo trdnejši in elastični. Obstaja tudi možnost pakiranja brez bojzani, da bi se špageti polomili.

Ob dodatku 2% psylliuma nismo opazili bistvenih razlik v izdelavi ter sušenjem testenin med ajdovo moko brez dodatkov ter ajdovo moko z dodatkom psylliuma. Testa vseh treh vzorcev so se obnašala približno enako kot brez dodatka. Prav tako so posušene testenine izjemno rahle in lomljive. Ugotavljamo, da dodatek psylliuma nima ustreznega tehnološkega učinka na boljšo elastičnost svežih testenin ter strukturo testenin po sušenju.

7 SKLEPI

- Raziskali smo možnosti za izdelavo testenin (špagetov) iz čiste ajdove moke (navadne ajde in tatarske ajde). Pripravili smo recepturo za izdelavo testenin iz ajdove moke in sicer razmerje moka in jajca 2 : 1.
- Ugotovili smo vidne razlike v barvi testenin med vzorci testenin iz navadne in tatarske ajde. Vzorci testenin iz tatarske ajde so bolj zeleno rumeno rjavi, medtem ko so ostali vzorci bolj svetlo rjavi.
- Ugotovili smo razlike v obnašanju testenin pri sušenju med posameznimi vzorci. Največje tehnološke težave se pojavijo pri izdelavi testenin iz tatarske ajdove moke.
- Pri dodajanju psylliuma v praškasti obliki v ajdovo moko med vzorci nismo ugotovili bistvenih razlik. Prav tako ni bilo razlik med vzorci z in brez dodatka psylliuma.
- Ugotavljamo pa vpliv guarjeve moke na elastičnost testenin, delno spremembo obnašanja testenin pri sušenju ter strukturo posušenih testenin. Testenine z dodatkom 5% guarjeve moke so bolj elastične, se manj lomijo pri sušenju ter posušene testenine so po strukturi obstojnejše in manj krhke, kar omogoča tudi pakiranje testenin.

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Agrovoc descriptors: soil water content; soil water potential; measurement; measuring instruments; equipment; methods

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Metode za merjenje količine vode v tleh 1. del: tenziometer

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IZVLEČEK

Znanje o količini vode v tleh ali v substratu je pri raziskovalnem delu na področju agronomskih in okoljskih znanosti kot tudi v praksi pri rastlinski pridelavi nujno potrebno za uspešno delo in zanesljive rezultate. Za merjenje vode v tleh poznamo direktne in indirektno metode določanja vsebnosti vode. Merjenje količine vode v tleh s pomočjo tenziometra spada med posredne metode, pri kateri merimo matrični potencial vode. Tenziometer je sestavljen iz porozne keramične kapice, povezane z manometrom preko največkrat rigidne cevke napolnjene z vodo. Tenziometri delujejo na območju okoli 80 - 85 kPa (teoretično do 100 kPa). Uporabni so za uravnavanje namakanja, ne pa tudi za meritve matričnega potenciala vode v zelo suhih tleh.

Ključne besede: merjenje vode v tleh, matrični potencial vode, tenziometer

ABSTRACT

SOIL WATER MEASUREMENT METHODS 1st part: TENSIOMETER

Soil water status is extremely important and necessary for successful work and reliable results in research in agronomy and environmental sciences as well as in practical applications in plant production. There are direct and indirect methods for soil water measurements. Measuring with tensiometers is an indirect method, based on water matrix potential measurements. Tensiometers consist of porous ceramic cup connected to a vacuum gauge through a rigid water-filled tube. Working interval is between 80 – 85 kPa (theoretically up to 100 kPa), which makes them useful for irrigation scheduling, but unsuitable for water matrix potential measurements in dry soils.

Key words: soil water measurement, soil water matrix potential, tensiometer

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1 UVOD

Tla so kompleksen sistem, sestavljen iz heterogene mešanice trdnih, tekočih in plinastih snovi. Trdno fazo predstavljajo mineralni delci in organska snov, ki so povezani v porozen prostor. V porah sta tekoča in plinasta faza tal. Tekočo fazo predstavlja talna raztopina, ki je največkrat voda s primesmi hranil in drugih snovi. Plinasto fazo tal predstavlja zrak, ujet v talne pore (Zupanc in Pintar, 2001; Pintar, 2006).

Za izračun vsebnosti vode v tleh, določitev lastnosti tal, ki vplivajo na tok vode v tleh ipd., uporabljamo razmerja med trdno, tekočo in plinasto fazo tal. V poroznem prostoru so pore izmenično zapolnjene s plinasto fazo oz. zrakom in tekočo fazo – talno raztopino oz. vodo (Zupanc in Pintar, 2001). Poznavanje koliko vode je v tleh ali v substratu, je pri raziskovalnem delu na področju agronomskih in okoljskih znanosti kot tudi v praksi pri rastlinski pridelavi nujno potrebno za uspešno delo in zanesljive rezultate.

Koliko vode se nahaja v porah tal, lahko izrazimo v masnem (g/g) ali volumskem (cm^3/cm^3) deležu. Uporaba volumskega deleža je ustreznejša od masnega, ker ga lahko neposredno vnesemo v izračune za količino vode, ki priteče v tla z dežjem ali namakanjem in se iz tal izgubi z evapotranspiracijo in drenažo. Volumsko razmerje je tudi enakovredno globinskemu razmerju tal, ki ustreza debelini plasti vode na enoto globine tal (Hillel, 1998; Adam, 2004).

Za merjenje količine vode v tleh poznamo direktne in indirektne metode. Metode se razlikujejo po zanesljivosti in ponovljivosti meritev, kako hitro so rezultati meritev uporabniku razpoložljivi, območjem meritev ter ceni opreme potrebne za meritve.

Pri direktni metodi (gravimetrična metoda) s sušenjem vzorca tal ali substrata (24 ur na 105°C za mineralna tla) neposredno določimo, kolikšno maso vode je vzorec vseboval. Gravimetrična metoda je splošno uporabljena kot standard posredne metode kljub njeni dolgotrajnosti in spreminjanju vzorca (destruktivnosti) tekom postopka meritve (Dirksen, 1999).

Pri posrednih metodah merjenja količine vode v tleh izkoristimo določeno lastnost trifaznega sistema tal (trdna snov – plin – tekočina). Prispevek opisuje merjenje količine vode v tleh s pomočjo tenziometra, ki spada med posredne metode merjenja količine vode v tleh. S tenziometrom količino vode izmerimo preko sile, s katero je voda vezana v tleh. Tenziometer torej ne izmeri, koliko vode je v tleh ali substratu, pač pa omogoča in situ meritve matričnega potenciala oz. t.i. tenzije vode v tleh (Hillel, 1998).

Prispevek opisuje merjenje količine vode v tleh s pomočjo tenziometra. Druge posredne meritve bomo opisali v drugem delu pregleda metod za merjenje količine vode v tleh.

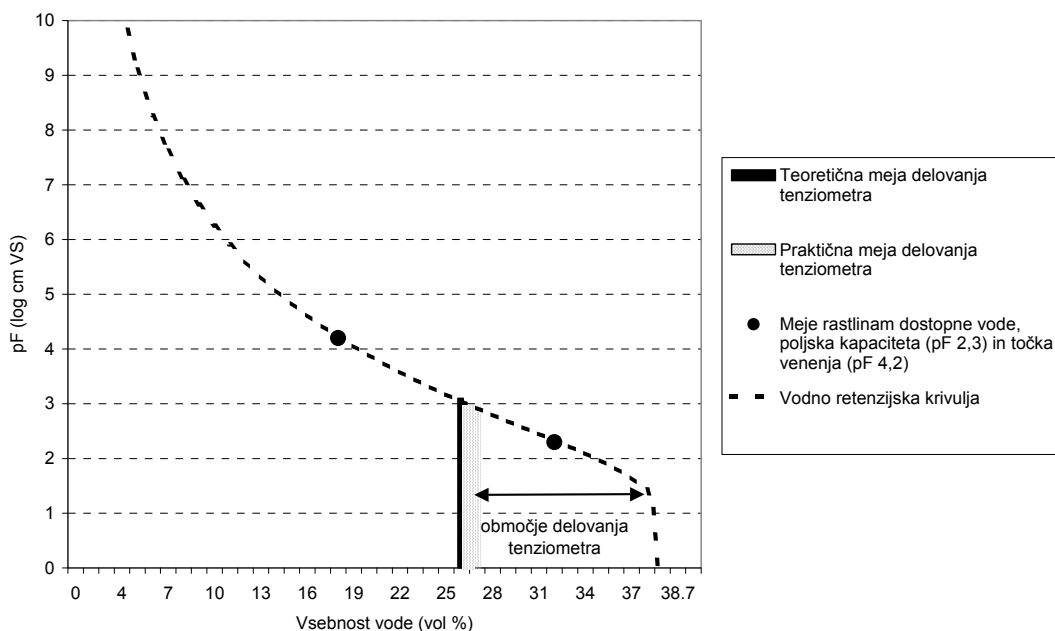
2 PRINCIP DELOVANJA

Potencialna energija talne vode je definirana kot delo, ki je potrebno za reverzibilni in izotermalni prenos masne enote vode iz danega referenčnega položaja na določeno točko v tleh. Vodni potencial je kazalec energijskega stanja vode. V nenasičeni coni (vadozna cona), kjer se nahaja večina korenin rastlin, določajo smer premika talne vode prostorske razlike matričnega potenciala vode.

Voda v tleh se na površino talnih delcev veže na dva različna načina. Prvi je adsorpcija molekul vode na površino talnih delcev preko Van der Valsovih sil. Drugi način je zadrževanje vode v tleh z menisknimi silami, ki se pojavijo na mestu kontakta dveh delcev (Hillel, 1998; Young in Sisson, 2002). Manjši je delež vode v tleh, večja je moč vezave. Matrični potencial je torej sestavljen iz adsorptivnih sil vode na talne delce ter kapilarnih sil znotraj por. Energija, potrebna za premaganje skupka teh sil, ustreza in je nasprotno enaka matričnemu potencialu. Tako ima matrični potencial vedno negativno vrednost v nenasičeni coni (Young in Sisson, 2002). Včasih so sile tenzije imenovali tudi kapilarni potencial. Ker pa je vezava vode na talne delce sestavljena tako iz adsorptivnih sil kot tudi kapilarnih – menisknih sil, so uvedli skupen pojem matrični potencial. To silo imenujemo sila vpijanja oz. sorpcije in jo v praksi večinoma izražamo v barih, pravilnejša pa je uporaba kPa (1 bar = 100 kPa). Ker govorimo o tenziji, je pred enoto negativen predznak. Tenzijo lahko izrazimo tudi v pF enotah (potential Force), ki so definirane kot negativni logaritem centimetrov vodnega stolpca (VS). Pretvorba 100 kPa oz. 1 bar ustreza višini 10 m VS oz. 1000 cm VS. Če želimo pretvoriti 100 kPa ali 1 bar, potem pretvorimo najprej v cm VS, te logaritmiramo, torej $\log 1000 \text{ cm VS}$, kar je 3 pF.

Energijsko ravnovesje med tenziometrom ter okoliškimi tlemi se doseže preko prehoda vode skozi porozno kapico tenziometra. Voda se giblje v smeri manjšega potenciala. Ko je matrični potencial vode v tleh okoli kapice manjši kot matrični potencial znotraj tenziometra, bo voda prehajala v okolico tal skozi pore kapice toliko časa, da se bo ustvarilo ravnovesje. Matrični potencial vode v okolici kapice ustreza podtlaku, ki se je pri ravnovesju ustvaril v tenziometru in ga odčitamo na merilcu podtlaka.

Podatek, ki ga preberemo na tenziometru, moramo s pomočjo krivulje tenzije (slika 1) spremeniti v količino vode, ki je v tleh (Pintar, 2006). Če želimo dobiti količino vode, moramo imeti vodno retenzijsko krivuljo za izbrana tla oz. substrat, ki ga uporabljamo v poskusu. Za pravi nadzor dodajanja vode je potrebno imeti tako meritve matričnega potenciala vode v tleh ali substratu, kot poznati vodnoretenzijske lastnosti tal ali substrata.



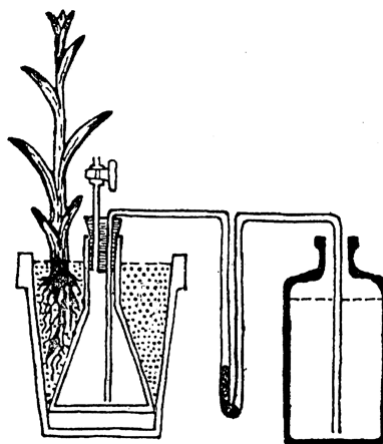
Slika 1: Primer poljubne vodno retenzijske krivulje (vsebnost vode v tleh v odnosu s silo vezave vode) ter na njej označeno območje delovanja tenziometra (0 – 3 pF).

Figure 1: Arbitrary water retention curve (water content vs. soil water suction) and the marked tensiometer working range (0 – 3 pF).

3 TENZIOMETER

Začetki tenziometrije segajo v zgodnje leto 1907, ko je Edgar Buckingham v svoji študiji *Studies on the movement of soil moisture* zapisal, da se sile, ki vežejo vodo v tleh lahko izražajo s kapilarnim potencialom. Če hočemo odstraniti iz tal vodo, ki je vezana v njih, je treba opraviti določeno delo (Buckingham, 1907). Izvirni načrt in obliko tenziometra je leta 1908 opisal Burton E. Livingston, kot sistem za uravnavanje vlage pri lončnicah (Slika 2). Splošno razširjeno in pogosto citirano uporabo tenziometra za merjenje matričnega potenciala vode v tleh pa je opisal Willard Gardner s sodelavci (1922). Prvi robustni načrt za uporabo na terenu pripisujejo Lorenzu A. Richardsu v zgodnjih dvajsetih letih dvajsetega stoletja (Or, 2001).

Tipičen tenziometer je sestavljen iz porozne keramične kapice, povezane z merilcem podtlaka preko največkrat toge cevke napolnjene z vodo (Slika 3). Keramična kapica je narejena iz keramike z zelo finimi porami. Služi kot vmesnik med vodo v tleh ter vodo v tenziometru. Prosto prepušča vodo, meniskus, ki je posledica površinske napetosti vode, pa do določene meje preprečuje vstop zraku. Ker je kapica v neposrednem stiku s tlemi, mora prenesti tudi obremenitve namestitve inštrumenta v tla v najrazličnejših razmerah (zbita, grobo peščena tla, ipd).

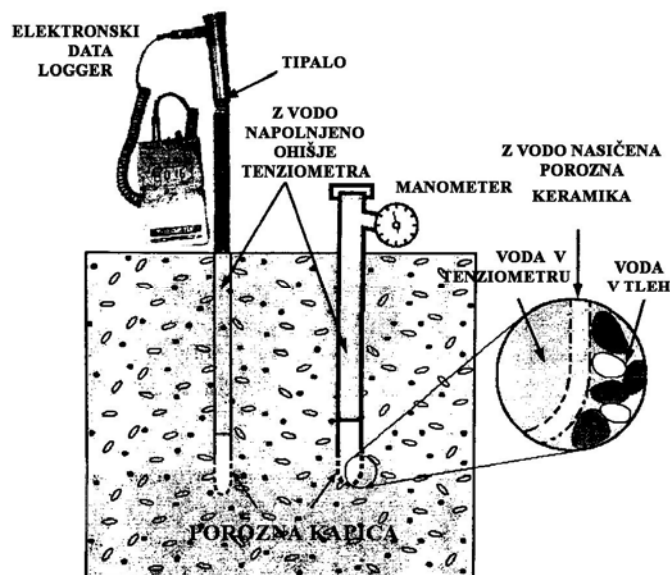


Slika 2: Skica Livingstonovega sistema za uravnavanje vlage v tleh (Livingston, 1908), ki vsebuje vse elemente modernega tenziometra za avtomatski nadzor vsebnosti vode v lončnem substratu (Or, 2001).

Figure 2: Livingston's design for self regulating system for soil water (Livingston, 1908), which uses all the elements of a modern tensiometer for automatically controlled soil water status of potted plants (Or, 2001).

Merilec za merjenje podtlaka, ki se ustvari v ohišju tenziometra, je lahko manometer ali elektronski prevodnik pritiska. Pri uporabi na terenu moramo upoštevati občutljivost merilca pritiska ter reakcijski čas.

Ohišje tenziometra je največkrat toga cevka. Vsi deli tenziometra so napolnjeni z vodo. Porozna kapica je v tesnem stiku s tlemi na globini oz. mestu, kjer želimo meriti vsebnost vode oz. tenzijo, s katero je voda v okoliških tleh vezana (Slika 3).



Slika 3: Skica tenziometra (levo z elektronskim prevodnikom pritiska, desno s klasičnim manometrom), nameščenega v talnem profilu (Or, 2001).

Figure 3: Tensiometer design (left with digital pressure transducer, right with classical manometer) in the soil profile (Or, 2001).

4 UPORABA APARATA

Tenziometer se uporablja v agronomiji in okoljskih znanostih predvsem za merjenje matričnega potenciala vode v tleh, prav tako se lahko uporablja za določevanje hidravličnih lastnosti tal, monitoring bogatenja podtalnice ter sledenje prenosa onesnažil. V praksi se tenziometri uporabljajo za uravnavanje statusa vode v tleh ali lončnem substratu z namakanjem. Tenziometri so preprosti za uporabo ter ne potrebujejo zunanjega napajanja z energijo (Young in Sisson, 2002).

Tenziometri so uporabni samo za podtlake do blizu 100 kPa (1 bar oz. 1 atm), to se pravi, da je mogoče izmeriti potenciale samo na vlažnem delu vodno retenzijske krivulje, to je od pF 0 do pF 3 (Kovačič, 1967). Rastlinam dostopna voda v tleh je med točko venenja (pF 4,2) ter poljsko kapaciteto (med 1,8 in 2,5 pF). To pomeni, da tenziometri merijo le v delu območja rastlinam dostopne vode. V tem razponu se gibljejo optimalne količine vode za sadje, vrtnine, poljska dela.

Praktične meritve potekajo do 85 kPa (2,97 pF). Ko je vsebnost vode v tleh na mejni točki, pride do vstopa zraka skozi pore keramične kapice. Posledica je znatno zmanjšanje podtlaka v cevki (ohišju) tenziometra. Meritve niso več skladne z dejanskim stanjem matričnega potenciala v tleh. Pri grobo zrnatih tleh se to lahko zgodi že prej, ko so tla še vlažna. Porozna kapica ima kontrolirano porazdelitev por tako, da lahko ostane nasičena, dokler matrični potencial okoli nje ne doseže 100 kPa (1000 cm VS).

Ozmotski potencial talne raztopine (npr. soli raztopljene v talni vodi) ne vpliva na meritve, ker se raztopljene snovi skozi porozno kapico lahko neovirano premikajo.

Ker delujejo na območju okoli 80 – 85 kPa (teoretično do 100 kPa), pomeni, da tenziometri niso uporabni za meritve matričnega potenciala vode v zelo suhih tleh. Prav tako niso primerni za poskuse, kjer se ugotavlja vpliv sušnega stresa, saj prenehajo delovati še v relativno vlažnem območju. Med in po sušnem obdobju jih je potrebno vzdrževati (cevno ohišje napolniti z vodo).

Pred namestitvijo na želeno merilno mesto v talnem profilu tenziometer namočimo v vodi. Če tenziometer nameščamo v zbita tla, predhodno vanje zavrtamo s svedrom, ki mora natančno ustrezati premeru kapice in ohišja tenziometra. Tenziometer s keramično kapico v tla namestimo previdno, tako da pri tem kapice ne poškodujemo in pazimo, da je stik s tlemi dober. Ko je tenziometer nameščen, tla vpijajo vodo skozi porozno kapico. Pri tem podtlak v tenziometru naraste, dokler podtlak v tenziometru ni v ravnotežju s matričnim potencialom vode v tleh okoli keramične kapice. Počakamo, da se stanje uravnovesi in odčitamo vrednost na barometru (Slika 4).

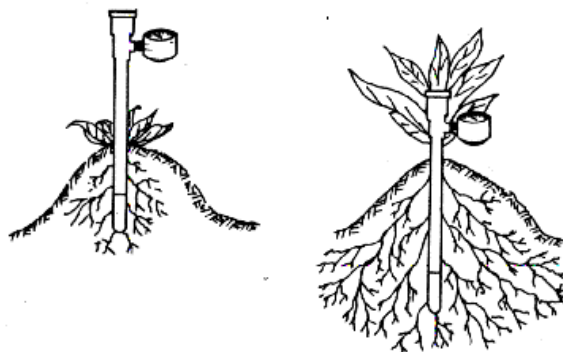
Meritve s tenziometrom so točkovne in nam podajo stanje vode v neposredni okolici korenin (Slika 5). V primeru, ko imamo rastline s plitvejšimi koreninami, za reprezentativen podatek zadošča uporaba posameznega tenziometra.



Slika 4: Primer dveh odčitkov na števcu manometra, levo dovolj vode, desno manj vode, kazalec se premika proti mejni vrednosti, ko tenziometer še deluje (85 kPa).

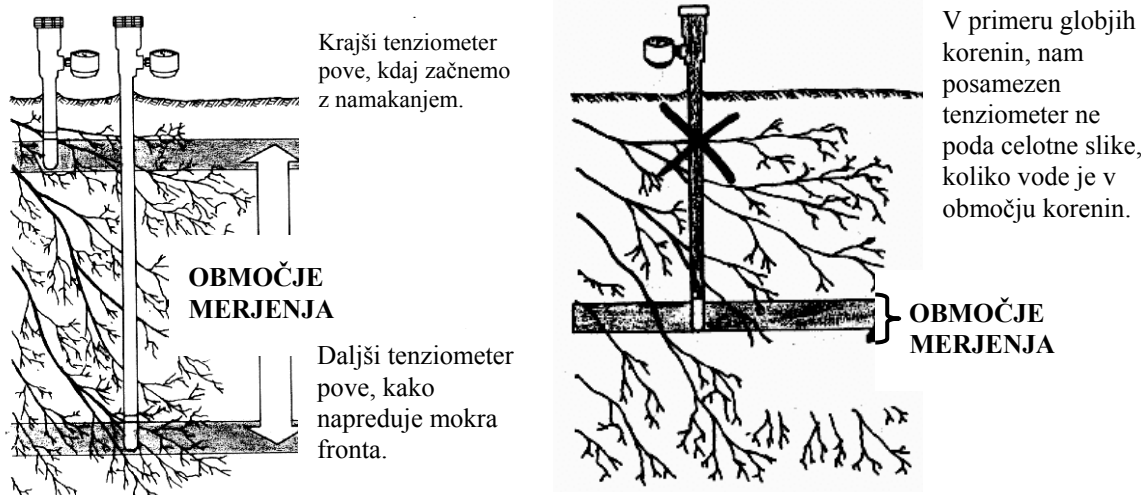
Figure 4: Example of two readings on manometer scale, left adequate water status, right low water status, cursor is moving towards boundary tensiometer working range (85 kPa).

Pri rastlinah z globljimi koreninami nam meritve z zgolj enim tenziometrom (Slika 6) ne povedo dovolj natančno, kakšno je stanje vode v tleh. Večina rastlin črpa glavino vode iz zgornje polovice do treh četrtin območja koreninskega spleta. Če želimo slediti gibanju vode, moramo uporabiti več tenziometrov, tako da lahko s pomočjo razlike v matričnem potencialu vidimo, v kateri smeri se giblje vodna fronta oz. ali je količina vode, ki smo jo v namakalnem obroku nanegli, dosegla želeno globino korenin. Priporočene globine za namestitev tenziometra glede na globine korenin različnih rastlin so v preglednici 1.



Slika 5: Uporaba tenziometra z enim tenziometrom na merilno mesto.

Figure 5: Tensiometer application in a one-instrument station.



Slika 6: Uporaba tenziometra z dvema oz. posameznim tenziometrom na merilno mesto (levo pravilno, desno nepravilno)

Figure 6: Tensiometer application with two or single tensiometer on measuring station (left correct, right wrong)

Preglednica 1: Globine namestitve tenziometra za merjenje količine vode v tleh (Tekinel in Čevik, 1993)

Table 1: Tensiometer installation depth for soil water measurements (Tekinel and Čevik, 1993)

Poljščina	Kratek tenziometer (cm)	Dolg tenziometer (cm)	Poljščina	Kratek tenziometer (cm)	Dolg tenziometer (cm)
zelje	30	50	čebula	30	45
nageljni	30	—	paprika	35	50
korenje	30	60	krompir	25	45
cvetača	30	60	sladek krompir	45	60
zelena	25	50	redkev	30	—
kumare	30	45	špinača	30	45
jajčevce	30	45	buče	35	50
česen	30	45	jagode	15	30
solata	30	—	paradižnik	30	45
melone	30	45	lubnice	30	45

4 ZAKLJUČEK

Razvoj tenziometra sega v začetek dvajsetega stoletja, izvorna oblika in princip te metode pa se je ohranila do danes. Zaradi preprostosti metode in cenovne dostopnosti so ena izmed najpogosteje uporabljenih naprav za uravnavanje količine vode v tleh.

Tenziometri so naprave, ki jih uporabljamo za merjenje matričnega potenciala vode. Delujejo v območju od 80 – 85 kPa (od zasičenega stanja z vodo do 2,9 pF), kar pomeni, da delujejo na mokrem območju vodno retenzijske krivulje in niso uporabni za ugotavljanje vodnega stresa oziroma merjenje statusa vode v suhih tleh.

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Agrovoc descriptors: landfills; leachates; leaching; boron; irrigation; waste disposal; urban wastes; salix; bioremediation; pollution control

Agris category codes: T01, P10, P30

COBISS code 1.01

Boron in irrigation water and its interactions with soil and plants: an example of municipal landfill leachate reuse

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ABSTRACT

In several countries, leachate is successfully treated by recirculation to the vegetated landfill cover, as it contains several micro and macronutrients for plant growth. However, the proportion and concentration of some parameters can negatively affect the plant growth and soil quality in the case of high leachate input. The presented research discusses B in leachate and its interactions with soil and plants. A ten-month field research was performed on 1.1 ha of the closed and covered municipal solid waste landfill site irrigated with landfill leachate. B concentration was analysed in leachate, landfill soil cover and in plant leaves. Total B concentration in leachate ranged from 0.8 to 3.83 mg/L. Monthly B mass load on the covered landfill site ranged between 0.2 – 1.5 kg/ha. The concentration of bioavailable B in soil cover gradually increased and ranged between <0.5 – 2.75 mg/kg dry weight soil. The average total B concentration in clover leaves was 23.9 mg/kg, in grass leaves 4.5 mg/kg, in the leaves of younger willows in the new part of the landfill cover 41.3 mg/kg and in the leaves of the older willows in the old part of the landfill cover 81.8 mg/kg. Leachate application increased plant growth during the observation period and there were no toxic effects on plant leaves, although B concentrations were higher compared to usual concentrations found in natural environment.

Key words: landfill leachate, municipal solid waste landfill site, boron, willow, irrigation, phytoremediation

IZVLEČEK

BOR V NAMAČALNI VODI IN NJEGOVE INTERAKCIJE S TLEMI IN RASTLINAMI: PRIMER PONOVI NE UPORABE IZCEDNE VODE ODLAGALIŠČA KOMUNALNIH ODPADKOV

V različnih državah izcedno vodo uspešno čistijo s pomočjo vračanja na vegetativno prekritje odlagališča, saj vsebuje več mikro in makrohranil za rast rastlin. Pri tem lahko razmerje in koncentracija nekaterih parametrov v izcedni vodi ob velikem vnosu v tla negativno vpliva na rast rastlin in kvaliteto tal. Predstavljena raziskava obravnava B v izcedni vodi ter njegove

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interakcije s tlemi in rastlinami. Deset mesečna erenska raziskava je bila opravljena na 1,1 ha zaprtega in prekritega odlagališča komunalnih odpadkov, ki je bilo namakano z izcedno vodo. Koncentracijo B smo analizirali v izcedni vodi, talnem prekritju odlagališča ter v listih rastlin. Celokupna koncentracija B se je v izcedni vodi gibala med 0,8 in 3,83 mg/L. Masni vnos B na prekritje odlagališča se je gibal med 0,2 in 1,5 kg/ha. Koncentracija biološko razpoložljivega B v tleh je zaradi vnosa izcedne vode postopoma naraščala in se gibala med <0,5 in 2,75 mg/kg suhih tal. Povprečna celokupna koncentracija B v listih detelje je znašala 23,9 mg/kg, v listih trave 4,5 mg/kg, v listih mladih vrb novega dela prekritja 41,3 mg/kg in v listih starejših vrb starega dela prekritja 81,8 mg/kg. Vnos izcedne vode je pospešil rast rastlin v času obdobja opazovanj. Na listih rastlin ni bilo opaziti strupenih učinkov kljub temu, da so bile koncentracije B višje v primerjavi z koncentracijami, ki jih običajno najdemo v naravnem okolju.

Ključne besede: izcedna voda, odlagališče komunalnih odpadkov, bor, vrbe, namakanje, fitoremediacija

INTRODUCTION

Landfilling is still the most common practice of municipal solid waste disposal. By the percolation of water through the waste, leachate is generated which represents substantial environmental concern (Kjeldsen et al., 2002; Dimitriou et al., 2006; Smith et al., 1999; Bowman et al., 2002; Maurice et al., 1999; Cureton et al., 1991). Environmental risk associated with uncontrolled landfill leachate outflow into the environment is largely attributed to the presence of high ammonium nitrogen levels, a range of organic substances, and of a high ionic strength caused by high concentrations of chloride and sodium. Conventional treatment of landfill leachate comprises co-treatment at municipal wastewater treatment plants or on-site leachate treatment in batch treatment processes, which require high energy and capital inputs and can generate large quantities of by-products. Landfill operators are therefore interested in local natural-based and low-cost treatment systems. In several European countries, Australia and USA, landfill leachate is treated by irrigation of recreational areas (Bowman et al., 2002), by recirculation to vegetated landfill cover (Maurice et al., 1999; Cureton et al., 1991) and by irrigation of energy crops (mainly short-rotation willow coppice) on restored parts of landfills or on adjacent arable fields (Dimitriou et al., 2006; Dimitriou and Aronson, 2004; Roseqvist and Ness, 2004; Nixon et al., 2000). Irrigation of leachate provides a mechanism of beneficial reuse of leachate with utilization of micro end macro nutrients from leachate for plant growth. Namely, leachate contains several components used by plants for the generation of biomass like water, macronutrients (N, K, Ca, Mg, and S) and micronutrients like B, Fe, Cu, Cl and Zn. Leachate can be therefore used as a substitute for water and inorganic fertilizers and thus reducing the need for non-renewable natural sources, which are being used for the production of fertilizers, although this *in-situ* phyto and bioremediation is limited by the capacity of the system to accommodate added pollutants. Of a particular concern are usually high concentrations of N and high salinity, especially in arid climates, while heavy metals are usually not of particular concern.

B is the tenth most abundant element in oceanic salts, varying in concentration in seawater from 0.52 mg/L in the Baltic Sea to as much as 9.57 mg/L in the Mediterranean Sea (Argust, 1998). Mean surface water B concentration ranges from

0.01 mg/L to 0.1 mg/L and for most groundwaters, the B concentration lies in the range 0.017 and 1.904 mg/L (Argust, 1989; EPA, 1991). Soil B concentration in general range from 2 to 100 mg/kg.

B presence and concentration in landfill leachate depend on the nature of deposited wastes. The main commercial applications are in the production of glass, textile fibreglass, ceramics, detergents, wood preservation materials, fire retardant materials, plastics, medicines, insecticides, and agricultural fertilizers (Argust, 1998; Sartaj and Fernandes, 2005). In addition, B may be present in other materials used in anthropogenic activities, such as fossil fuel burning. The use of B in the manufacture and the use of products in these industries lead to a wide diffusion of B into the environment through various pathways and ending in waste material also on landfill sites.

B causes problems especially in irrigation water. It is an essential micronutrient for higher plants, with interspecies differences in the levels required for optimum growth. The extent to which B is present in different plants and animals varies a great deal, from <0.07 mg/kg B in animal livers up to as high as 248 mg/kg B in some seaweeds (Argust, 1998). There is a small range between B deficiency and toxicity in some plants (WHO, 1998; Gupta et al., 1985; Sartaj and Fernandes, 2005). Francois and Clark (1979) investigated the B tolerance in 25 ornamental plants of which about 15 were moderately damaged by a B concentration of 2.5 mg/L and severely damaged or killed at B concentration in the irrigation water of 7.5 mg/L. Safe concentrations of B in irrigation water range from 0.3 – 1.0 mg/L for sensitive plants (avocado (*Persea americana*), apple (*Malus domestica*), bean (*Phaseolus vulgaris*)), 1 – 2 mg/L for semi tolerant plants (oat (*Avena sativa*), maize (*Zea mais*), potato (*Solanum tuberosum*)), and 2 - 4 mg/L for tolerant plants (carrot (*Daucus carota*), alfalfa (*Medicago sativa*), and sugar beet (*Beta vulgaris*)) (Keren and Bingham, 1985; Nable et al., 1997). By Ayers and Westcot (1994), there are no restrictions on the use of irrigation water foreseen at the B concentrations <0.7 mg/L, at the concentrations between >0.7 and 3.0 mg/L slight to moderate restriction on the use is set, and at B concentration > 3.0 mg/L severe restriction on the use of irrigation water is foreseen. Referred to Nable et al. (1997), the present knowledge is insufficient to define precisely the acceptable B levels in a growth medium, but soils containing more than 5 to 8 mg/L of hot H₂O soluble B may require special revegetation considerations. However, many native species are well adapted to B levels in excess of 5 mg/L.

Slovenian legislation sets limits for B concentration in the discharge of wastewater into waters (1 mg/L) and public sewage system (10 mg/L) (OG RS, 47/2005). The limit B concentration in drinking water is 1 mg/L (OG RS, 19/2004). The maximum concentration of B in the irrigation water is not defined by Slovenian legislation (OG RS, 84/2005). However, the use of wastewater for irrigation from different sources is not a usual practice in Slovenia, as there are for now satisfactory amounts of surface and underground water. With increased occurrence of summer drought and directions toward recycling and reuse of usable wastes there is a need also in Slovenia to approach toward the possibilities of sustainable use of wastewater sources as a possible reusable nutrients and water sources.

B is one of the most troublesome trace elements in soil management, as it is adsorbed to soil constituents as well as being present in slowly soluble form. B is mainly found in soil in the form either of boric acid (H_3BO_3) or calcium and sodium borates. Irrigation of soil with high B water can lead to the incorporation of B into sites not readily desorbed (carbonate, organic, and free ion portions), which can lead to significant accumulation of B in a period of time on the order of a decade. Continued irrigation with B laden water will exceed the adsorption capacity of the soil and B will accumulate in soil and cause a possible reduction in crop yield (Nable et al., 1997). The primary loss of B from the soil is leaching. Leaching is also the technique used to remove excess B from the surface soil and the root zone, although B leaching is approximately four times slower than sodium (Sartaj and Fernandes, 2005). The situation is therefore more serious in arid and semi-arid environments with low rainfall or where water used for leaching is unavailable.

B absorption by plant roots is affected by various environmental factors, both in the soil and non-soil environments. Important factors influencing B absorption from solution include the initial B content of the soil, the pH of the soil, the type of exchangeable ions present in the soil, the soil organic matter content, the wetting and drying cycles, and the water to soil ratio (Gupta et al., 1985; Romero and Aguilar, 1986; Hu and Brown, 1997). Humidity seems to affect B availability stronger than for other elements, indicating that B insufficiency in plants during drought may be partially associated to the level of B soluble in the water in soil. Studies on B interactions showed that N is the most important as far as its effect on B absorption by plants is concerned (Romero and Aguilar, 1986). Studies showed that B concentrations decreased with increasing quantities of N and that additions of N decreased the toxicity symptoms of B. It seems that B can have positive influence on the absorption of other micronutrients like Zn, Mo, Mn, and Cu (Romero and Aguilar, 1986).

Physiologically, B is considered as an important element for the development of plant tissues (Romero and Aguilar, 1986; Nable et al., 1997). The rapid and specific inhibition of plant growth that occurs upon removal of B is a consequence of two important features of B physiology: the specific structural role of B in the cell wall and the limited mobility of B in the majority of species (Hu and Brown, 1997). As a result of its critical role in expanding tissues and its limited mobility, B has to be supplied continually throughout the life of the plant (Hu and Brown, 1997).

A typical visible symptom of B toxicity is leaf burn – chlorotic and/or necrotic patches, often at the margins and tips of older leaves (Nable et al., 1997). These symptoms reflect the distribution of B in most species, with B accumulation at the end of the transpiration stream (Hu and Brown, 1997; Nable et al., 1997). Tissue concentrations of B can vary considerably; therefore, it is difficult to use foliar analysis for diagnosis of B toxicity (Nable et al., 1997). In species that accumulate B in their leaves, these tissues normally contain about 40 to 100 mg/kg of B dry wt. However, the leaves can contain 250 mg/kg dry wt when B in the soil approaches toxic levels. Leaf concentration of B may exceed 700 to 1,000 mg/kg dry wt in extreme conditions of B toxicity.

In this paper we would like to expose the problems of B from landfill leachate in the case of leachate recirculation to the vegetated landfill cover. A ten month field research was performed at the municipal solid waste landfill site at Dobrava near Ormož in the southeastern part of Slovenia. 1.1 ha of the closed and covered landfill site was irrigated with landfill leachate by underground irrigation system placed on 30 cm depth. The landfill site was covered with 1 m soil layer and planted with willows (*Salix sp.*), grass mixture and white clover (*Trifolium repens*). The covered part of the landfill site was divided into two parts according to the landfill closure steps. The first part (old cover) has been planted and irrigated since 2003 and the second part (new cover) since 2004.

MATERIALS AND METHODS

Total B concentration in leachate was monthly analysed from grab samples taken from the reservoir from where leachate had been pumped on the landfill cover. Before the determination of total B concentration in leachate, triplicate samples were wet digested in Kjeldahl flasks using HNO_3 and H_2O_2 .

Soil core samples were collected four times per year from 25 evenly distributed spots, separately on the new and old plantation, using the soil probe. The soil cores were taken at the depth of 90 cm and divided into three parts (0-30 cm, 30-60 cm and 60-90 cm). The cores from the same depths were mixed, air dried, sieved through 2 mm sieve and ground to powder. The total B concentration in soil was determined after *aqua regia* ($\text{HNO}_3:\text{HCl} = 1:3$) digestion of samples. To evaluate a bioavailable B fraction, the extraction of soil samples in 0.11 M CH_3COOH was performed (Rauret et al., 2000).

Plant samples were collected at the end of the growing season, including leaves of willows, grass and clover. Microwave-assisted total digestion of plant samples was performed by $\text{HNO}_3:\text{HClO}_4 = 7:1$ extraction (Sastre et al., 2002).

Digested diluted samples of leachate, soil and plants were analyzed with Agilent 4500 series ICP-MS instrument (Babbington nebulizer with Peltier-cooled spray chamber, carrier gas Ar, 1.05 L/min, RF power 1300 W). Standard addition technique ($N = 10$) was used to avoid matrix interferences. The limit of detection was calculated as a concentration corresponding to three-fold standard deviation ($3s$, $N = 6$) of blank determinations. Blanks were subjected to the same digestion procedure as samples.

TraceSelect (Fluka, Buchs, Switzerland) and Suprapur (Merck, Darmstadt, Germany) acids and deionised water (Milli-Q) were used for the preparation of samples, extraction solutions and standard solutions.

The data on precipitation, temperature and potential evapotranspiration were acquired from the nearby weather station at Jeruzalem. The amount of pumped leachate was followed by the pump counter.

Pedologic analyses were performed according to standards: SIST ISO 10390:1996 (pH of soil samples), SIST ISO 14235:1999 (organic matter content), and SIST ISO 11260:1996 (cation exchange capacity - CEC).

RESULTS AND DISCUSSION

Leachate

From January to October 2005, the concentration of B in landfill leachate recirculated to the planted landfill cover by underground irrigation system ranged between 0.8 and

3.83 mg/L (Figure 1). Varying concentrations of B in landfill leachate are reported, ranging from 0.3 – 63.2 mg/L (Castonguay et al., 1996), 2.6 – 4.0 mg/L from municipal solid waste landfill in Florida (Statom et al., 2006), 0.05 – 1.1 mg/L from small closed New Zealand landfills and 1.1 – 10 mg/L from large operational New Zealand landfills (Golden Associates, 2002), 10.5 mg/L in average from Ontario landfill (Sartaj and Fernandes, 2005), to up to 64 mg/L from municipal solid waste landfill in Ljubljana, Slovenia (Bulc, 1998). Compared with the data from the literature, leachate from the landfill under consideration was not excessively loaded with B. However, according to the normative values laid down by Decree on the Emission of Substances and Heat in the Discharge of Wastewater into Waters and Public Sewage System (OG RS, No. 47/2005), the values would be exceeded in the case of its discharge into a watercourse (normative W – 1 mg/L), but not if discharged into the sewage system (normative S – 10 mg/L). As leachate was reused and recirculated to the vegetated part of the landfill with an isolated base, there was no discharge of leachate into the environment and, therefore, no pollution of surface and underground waters.

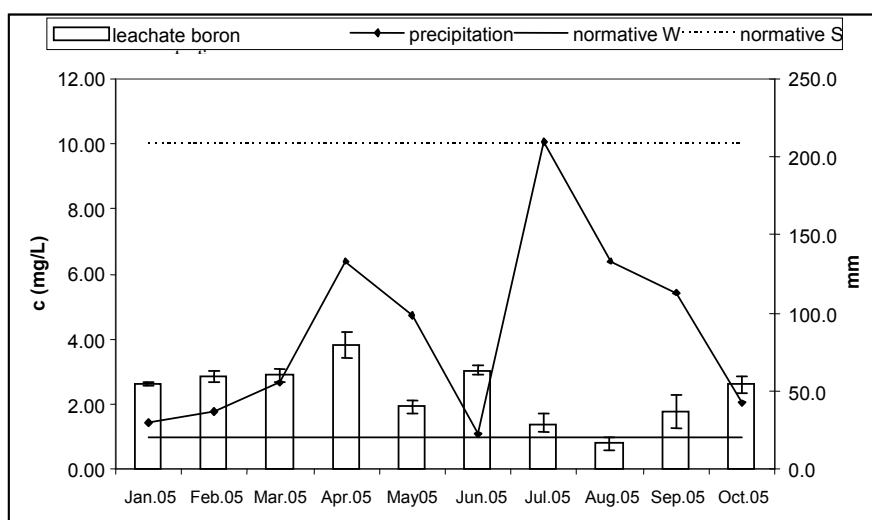


Figure 1. Monthly measurements of B in leachate used for irrigation. Maximum permissible concentration of B in waste water is presented for the outflow into surface waters (normative W) and the sewage system (normative S) (OG RS, 47/2005). Cumulative monthly amount of precipitation (mm) is calculated between monthly leachate sampling intervals.

Comparing the measured concentration of B in leachate and the limit values or safe B concentrations in irrigation water (Keren and Bingham, 1985; Nable et al., 1997; Ayers and Westcot 1994), the water used in this case was at the medium limit level (<0.7 – 3 mg/L). The recommendations cited and the restrictions on B concentration in irrigation water apply to crops and the use of water in dry climates where irrigation water is the main source of water during high water deficit in growing season. In our case, the leachate input represented 28% of total water received by a square metre of surface during the growing season, i.e. from April to September and 37.5% during the monitoring period from January to October 2005, respectively (Figure 2). This means that the actual concentration of B in water was lower, except during the time of high evapotranspiration and water deficit. In the event of longer drought periods, when B

concentration in soil solution is higher due to evapotranspiration, according to the literature, the concentration of B in leachate may be toxic to plants. Monthly mass load of B through leachate per 1.1 ha of vegetated landfill site ranged from 0.2 to 1.5 kg/ha or, on average, 0.8 kg/ha (Figure 2). Total mass load during the monitoring period from January to October 2005 was 7.9 kg/ha.

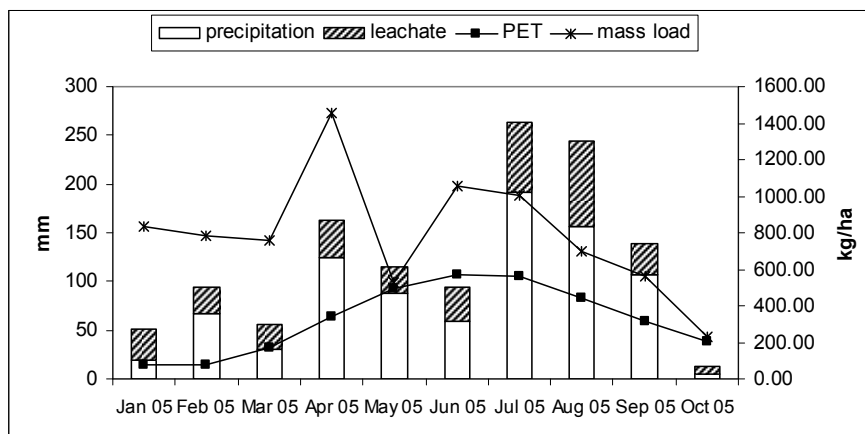


Figure 2: Cumulative monthly amount of B mass load (kg/ha) with leachate irrigation, monthly amount of precipitation (mm) and leachate water (mm) irrigated on 11,000 m² of planted area with potential evapotranspiration (PET, mm).

Soil

Only part of the total quantity of metals in soil may be considered bioavailable, labile, mobile and potentially toxic. To determine easily soluble metal fractions available to plant uptake, water and electrolyte solutions, e.g. acetic acid, were commonly used (Rauret et al., 2000). Soil water solution is prepared to estimate the proportions of metals that can be transferable into water and therefore easily available to plants. Solutions with acetic acid are prepared to estimate the proportions of metals not easily water soluble, but bioavailable as plants excrete similar compounds which increase metal solubility. In our research, total and acetic acid extractable (bioavailable) B concentrations in soil cover samples were measured.

The results of measurements of total and bioavailable B concentrations showed that all B in soil was in bioavailable form (the difference between total and bioavailable B concentration of the same sample was in the range of analytical error), so only the results of bioavailable B determination are represented (Figures 3 and 4). At first sampling in January 2005, only bioavailable B concentration in upper soil layer (0-30 cm) of the new cover exceeded the LOD (0.5 mg/kg). During this period, the amount of precipitation was much lower than the amount of leachate recirculated to the planted landfill cover (Figure 2). At the second sampling, bioavailable B concentrations in all soil samples were below LOD in spite of continuous irrigation of the soil cover with leachate. Obviously, due to precipitations, the leaching of B in the first part of the year was sufficient to keep B concentration at such a low value. From the third sampling in August 2005, bioavailable B concentrations began to increase and thus higher B concentrations were measured on the new cover. Higher soil B concentrations in the new cover could be explained by lower plant uptake and lower

evapotranspiration. Willows on the new cover were less developed than willows on the old cover where they reached the height of 2 m. Beside this, the average B concentration in the willow leaves of the old cover was higher than the B concentration of the willow leaves on the new cover. Predominant vegetation on the new cover were grasses and white clover. Referred to Reinmann et al. (2001), birch and willow are very successful in supplying themselves with major (S, P, K, Ca and Mg) and minor (B, Cu and Zn) nutrients, where plant/soil bioaccumulation ration for B in willow leaves reached 6.4. As can be seen from Figures 3 and 4, the concentration of bioavailable B on the new and old cover decreased with depth. As the irrigation system was placed on the 30 cm depth, the highest B concentration was therefore expected to be on the depth between 0 and 60 cm and the lowest on the depth of 90 cm.

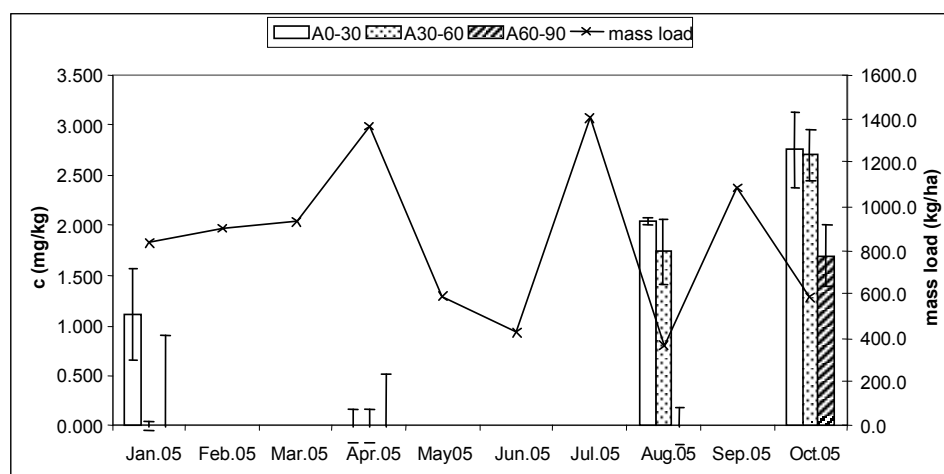


Figure 3: Time variation of bioavailable B concentration (mg/kg) in three soil layers (0–30, 30–60, 60–90 cm) on the new landfill cover (A) with monthly B mass load (kg/ha) calculated between monthly leachate sampling intervals.

Within the soil system, a variety of mechanisms exist that will influence the resistance time of B within the soil. These include the relative demand for B from plant life within the soil, the amount of rainfall either washing B out of the soil profile or adding B to it, the action of clays acting as adsorption/desorption sites for B, temperature, organic matter and soil pH (Argust, 1998; Sartaj and Fernandes, 2005). The pH of soil is one of the most important factors affecting the B availability in soil and plants (Romero and Aguilar, 1986), with higher availability to plants with decreasing pH. The pH of our soil samples were not changing considerably during the measuring period and it varied from 7.2 to 7.6, indicating that pH did not have an important role in the B uptake by plants. The second important factor, which affects B availability, is the soil texture (Romero and Aguilar, 1986). In the soils with fine texture (clay), larger quantities of B soluble in hot H₂O were found than in soils with coarse texture, indicating that B is adsorbed in clay particles. However, the soil with high adsorption capacity will maintain lower B concentration in soil solution. In coarse textured soil low in clay and low in organic matter, B is highly mobile and a subject to leaching losses. Increasing of B concentration in soil samples in our case indicates an important influence of adsorption of B onto soil particles. The soil on the covered part of landfill consisted of 56% of silt fraction, 23% of clay fraction and 21% of sand fraction with the CEC amount from 16.5 to 24.7 cmol/kg, which enabled

B adsorption. On the other hand, the organic matter content of soil was low and varied from 0.6 to 1.2 and therefore not contributing to additional B accumulation.

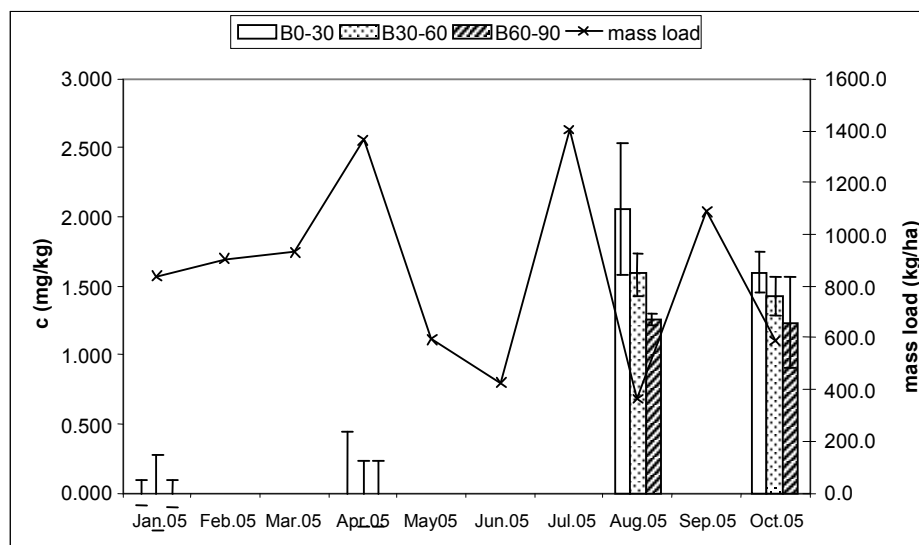


Figure 4: Time variation of exchangeable B concentration (mg/kg) in three soil layers (0–30, 30–60, 60–90 cm) on the old landfill cover (B) with monthly B mass load (kg/ha) calculated between monthly leachate sampling intervals.

Plants

The average B concentration in clover leaves was 23.9 mg/kg, in grass leaves 4.5 mg/kg, in the leaves of younger willows in the new part of the landfill cover 41.3 mg/kg and in the leaves of the older willows in the old part of the landfill cover 81.8 mg/kg. On natural sites, B concentration in the leaves of different plants varies considerably. Bargagli (1998) states the following typical B concentrations (mg/kg dw) in the leaves of different plants: apple tree 27, peach tree 2, spinach 37.6, citrus 21, tomato 33.3, *Lolium perenne* 6.2, *Taraxacum officinale* 10, *Robinia pseudoacacia* 15, wheat 4.9, barley 9. Reimann et al. (2001) measured on average 15.9 mg B/kg dry wt on 23 samples of willow leaves (*Salix* sp.) in the area of 1,500,000 km² in northern Europe, whereas Alker et al. (2002), after three years of irrigation by leachate, measured the B concentration in willow leaves of between 11.3 – 44.6 mg/kg, while the concentration in control plants, which were not watered by leachate, ranged from 5.0 to 11.7 mg/kg. In our case, the B concentrations in grass and willow leaves were higher in comparison with the concentrations under natural conditions (Bargagli, 1998; Reimann et al. 2001). Compared with Alker et al. (2002) results, higher B concentrations were measured in the leaves of the older willows in the old part of the landfill. Moreover, our measurements were carried out in September, at the end of the growing season, while Alker's data refer to the measurements made in August. In their research, Alker et al. (2002) found out that irrigation by leachate from the municipal landfill enhanced the plant growth as compared with the part, which was irrigated only by water, and the part where no water was added. The findings of our research were the same. The introduction of leachate into the soil of landfill cover resulted in a more vigorous growth of vegetation and faster burst into leaf in spring in comparison to ruderal vegetation growing next to the landfill on the soil of the same composition as of the landfill cover layer. Also, there were no visible symptoms of leachate toxicity on leaves, such as chlorotic or necrotic patches. This finding indicates that the measured higher B values in leaves were not toxic to plants.

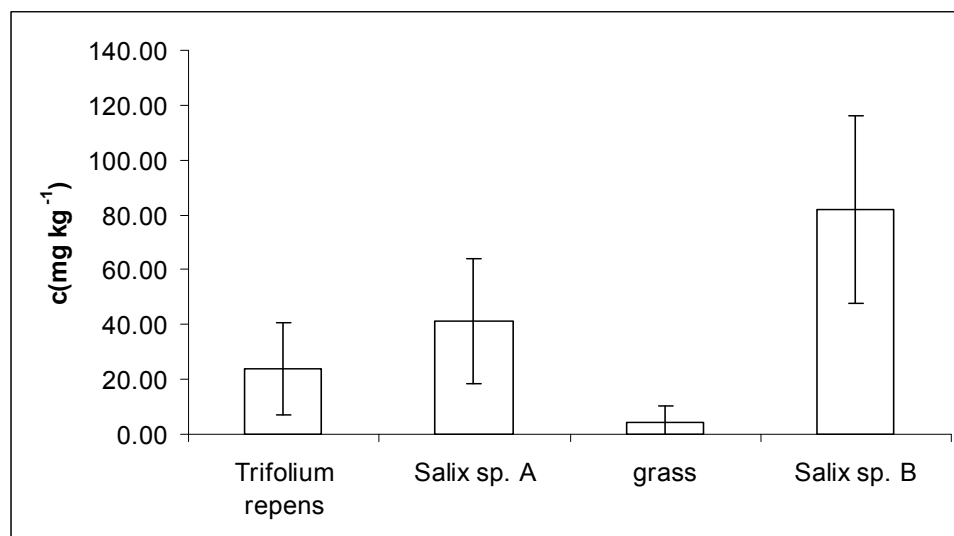


Figure 5: B concentration (mg/kg) in the leaves of white clover (*Trifolium repens*), willow (*Salix sp.*) and the grass mixture irrigated with landfill leachate.

During the monitoring period, the identified increase of B in soil contributed to better supply of the soil with B. In the event of further increase in B concentration in soil, could lead to the possibility of toxicity; for that reason, there is a need for longer monitoring of changes in B concentration in the soil and plants. In addition, leachate is a complex mixture of organic and inorganic substances. Therefore, the plant uptake of B does not depend only on the concentration of bioavailable B in soil solution, but also on the interaction with other leachate components.

CONCLUSIONS

During the ten-month monitoring period, the use of landfill leachate with B concentration at the medium limit level, applicable to the irrigation water in agriculture (Ayers and Westcot, 1994), did not have negative effects on the growth of landfill cover vegetation consisting of grass mixture, white clover and willows. However, there was a distinctive trend of an increased concentration of bioavailable B in the soil as well as increased B concentration in plant leaves as compared to the typical B concentrations in plants on natural sites. Because of the identified increasing trend in B concentration the monitoring is being continued to identify long-term change dynamics of B in soil cover and plants. Considering the high intake rate of B by willows, there is a possibility of efficient removal of B by regular cutting of vegetation, as the removal of B from leachate by traditional methods is difficult. Thriving of plants at higher B concentrations indicates that less severe restrictions regarding B concentration in irrigation water are necessary for planting of ligneous vegetation than for crops in temperate climates.

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CONTENT ANALYSIS OF THE PAPERS IN THE RESEARCH REPORTS vol. 89, no. 1

VSEBINSKA OBDELAVA PRISPEVKOV V ZBORNIKU let. 89, št. 1

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NAVODILA AVTORJEM

Prispevki

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Pri prispevkih v slovenskem jeziku morajo biti preglednice, grafikoni, slike in priloge dvojezični, povsod je slovenščina na prvem mestu. Naslovi grafikonov in slik so pod njimi. Slike in grafikoni so v besedilu. Priloženi morajo biti tudi jasno označeni izvorniki slik. Na avtorjevo željo jih vračamo, s tem da je želja pisno sporočena ob oddaji gradiva in ponovno v teku 30 dni po izidu. Latinske izraze pišemo ležeče. V slovenščini uporabljamo decimalno vejico, v angleščini decimalno piko. Prispevki v angleščini morajo imeti povzetek v slovenščini in obratno. Prispevki v nemščini morajo imeti tudi povzetka v slovenščini in angleščini.

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Prispevke sprejemamo vse leto.

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